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1. SHAPIRO, S., WEINER, M., ET AL.: AM. HEART J. 40:766 (NOV.) 1950.

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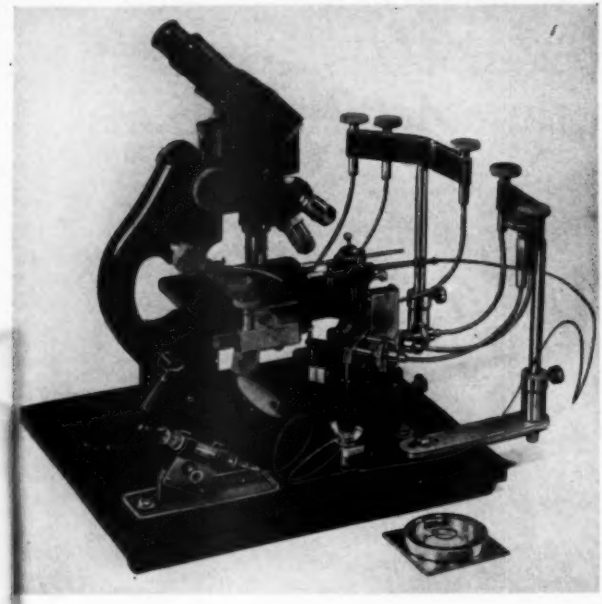

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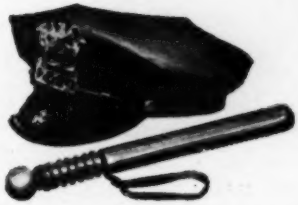

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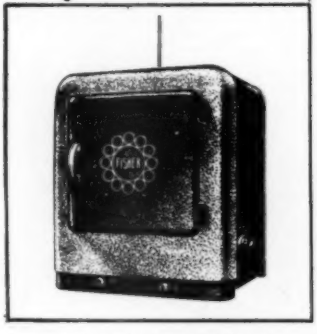

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ARCHIVES OF PATHOLOGY

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# VASCULAR LESIONS IN ALLOXAN DIABETIC RATS

A. L. CHUTE, M.D.
J. L. ORR, B.A.
M. J. O'BRIEN, M.D.
AND
E. E. JONES, M.D.
TORONTO, CANADA

IN A RECENT address, Harrison [1] emphasized the increasing importance of vascular disease as a cause of death.

A particularly high incidence of degenerative vascular disorders is seen in association with certain metabolic diseases, such as diabetes mellitus.[2] For this reason, the study of vascular changes in experimentally induced diabetes was deemed worthy of investigation.

## METHODS

Rats [3] weighing 100 to 150 gm. were starved 48 hr. and were then given by subcutaneous injection 200 mg./kg. of a freshly prepared 2% aqueous solution of alloxan [4] (Eastman Kodak Company). They were fed a standard diet (Diet 2), and after a two to four week period those animals which still had elevated fasting blood sugars and glycosuria were selected for experimentation. This preliminary period enabled us to exclude most of those animals which failed to become permanently diabetic.[5] The animals were then divided into the following groups for each experiment:

A. Diabetic animals on a weighed diet, receiving no insulin.

B. Diabetic animals on a weighed diet, receiving insulin.

C. Normal animals on an ad libitum diet.

---

This research was supported by a grant from The Canadian Life Insurance Officer's Association.

From the Wards and Laboratories of the Hospital for Sick Children and the Department of Paediatrics, University of Toronto, under the direction of Alan Brown, M.D., F.R.C.P. (C), F.R.C.P. (Lond.).

1. Harrison, T. R.: Association of Life Insurance Medical Directors of America **32**:12, 1948.

2. Chute, A. L.: Survey of Patients with Juvenile Diabetes Mellitus, Am. J. Dis. Child. **75**:1 (Jan.) 1948. Dolger, H.: Clinical Evaluation of Vascular Damage in Diabetes Mellitus, J. A. M. A. **134**:1289 (Aug. 16) 1947. John, H. J.: J. Pediat. **35**:723, 1949. White, P., and Waskow, E.: South. M. J. **41**:561, 1948.

3. These rats were white rats inbred for approximately 20 years in colony at the Hospital for Sick Children, Toronto.

4. Dunn, J. S., and McLetchie, N. G. B.: Lancet **2**:384, 1943. Dunn, J. S.; Sheehan, H. L., and McLetchie, N. G. B.: Ibid. **1**:484, 1943.

5. Cantor, M. M.; Tuba, J., and Capsey, P. A.: Science **105**:476, 1947. Lazarow, A.: Proc. Soc. Exper. Biol. & Med. **61**:441, 1946. Wrenshall, G. A.; Collins-Williams, J., and Hartroft, W. S.: Am. J. Physiol. **156**:100, 1949.

D. Diabetic animals on an ad libitum diet, receiving no insulin.

E. Diabetic animals on an ad libitum diet, receiving insulin.

F. Alloxanized animals which failed to become diabetic on an ad libitum diet.

In all experiments the animals in Groups A and B were pair-fed with the control animals of Group C.

The animals were placed in individual metabolic cages, permitting the estimation of weekly sugar excretion and 24-hr. protein excretion. Blood sugar and nonprotein nitrogen levels were checked from time to time. Indirect blood pressure measurements were made by using the Sobin[6] modification of the method of Williams, Harrison and Grollman.[7] Animals from each group were sacrificed at intervals, and a gross and a microscopic examination of their tissues and blood vessels were undertaken.

## EXPERIMENTS AND RESULTS

*Experiment 1* (Standard Diet).—Twelve animals in each of four groups, A, B, C, and D, were fed either a 50%-carbohydrate diet[8] (Diet 1) or a 55%-carbohydrate standard diet (Diet 2). The animals were sacrificed at intervals varying from eight to 52 weeks after alloxanization.

*Experiment 2* (High Fat Diet—36%).—Five animals in each of four groups, A, B, C, and D, were fed a 36%-fat diet (Diet 3) and sacrificed at intervals varying from six to 49 weeks after alloxanization.

*Experiment 3* (High Protein Diet—40%).—Five animals in each of four groups, A, B, C, and D, were fed a 40%-protein diet (Diet 4) and sacrificed at intervals varying from 12 to 50 weeks after alloxanization.

*Experiment 4* (2%-Cholesterol Diet).—Four animals in each of five groups, A, B, C, D, and E, were fed a standard diet modified to contain 2% cholesterol (Diet 5) and sacrificed at intervals varying from 17 to 34 weeks after alloxanization.

Aside from the development of cataract in a large number of the diabetic animals,[9] no distinctive changes were observed in any of the animals in experiments 1 to 4, nor was there any manifestation of vascular disease.

*Experiment 5* (10%-Sodium Chloride Diet).—In this experiment rats were fed a standard diet modified to contain 10% by weight sodium chloride (Diet 6). In 38% of the animals in group A, 33% in group B, 75% in group D, 80% in group E, and 50% in group F, vascular lesions developed (Table 1), the nature of which will be discussed later. None of the animals in the nonalloxanized control group (Group C) showed evidence of vascular disease.

The time of appearance and the severity of the vascular lesions varied so greatly that no correlation could be demonstrated between these changes and the effect of controlled diet, insulin, or duration of diabetes.

In a number of cases the blood nonprotein nitrogen was elevated, and the ratios of $\frac{\text{heart weight}}{\text{body weight}}$ and $\frac{\text{kidney weight}}{\text{body weight}}$ for the alloxanized groups were greater than those for controls of similar weights.

Fifty per cent of alloxanized animals which failed to become diabetic (Group F) also showed typical vascular changes. These lesions were not seen in a similar group of alloxanized, nondiabetic animals (Experiment 1, Group F) fed the same diet without added sodium chloride.

*Experiment 6* (5%-Sodium Chloride Diet).—In this experiment rats were given a standard diet modified to contain 5% by weight sodium chloride (Diet 7) and were killed at intervals varying from 11 to 32 weeks after alloxanization. The number of animals in each group was similar to that in experiment 5. The results were similar to, though less pronounced than,

6. Sobin, S. S.: Am. J. Physiol. **146**:179, 1946.

7. Williams, J. R., Jr.; Harrison, T. R., and Grollman, A.: J. Clin. Invest. **18**:373, 1939.

8. The diet used was purina® fox chow—Diet 1. The details of all the diets may be found in the table in the Appendix.

9. Bailey, C. C.; Bailey, O. T., and Leech, R. S.: New England J. Med. **230**:533, 1944.

TABLE 1.—*Experiment 5*

| Rat | Sex | Duration of Diabetes, Weeks | Duration of Diet 6, Weeks | Terminal Blood Nonprotein N, Mg./100 Cc. | Terminal Weight, Gm. | Organ Wt./Body Wt. × 10⁴ Kidney (Av.) | Organ Wt./Body Wt. × 10⁴ Heart | Protamine Zinc Insulin, Units/Day | Urine Sugar, Gm./Week | Evidence of Renal Vascular Disease |
|---|---|---|---|---|---|---|---|---|---|---|
| **Group C** | | | | | | | | | | |
| 1 | F | .. | 17 | 68 | 230 | ... | ... | ... | .. | Negative |
| 7 | M | .. | 11 | 50 | 120 | ... | ... | ... | .. | Negative |
| 9 | F | .. | 12 | 48 | 120 | ... | ... | ... | .. | Negative |
| 11 | F | .. | 32 | 98 | 240 | 4.6 | 3.5 | ... | .. | Negative |
| 3 | F | .. | 22 | 73 | 240 | 5.0 | 3.8 | ... | .. | Negative |
| 5 | M | .. | 28 | 53 | 320 | 4.0 | 4.1 | ... | .. | Negative |
| 12 | F | .. | 32 | 138 | 220 | 5.7 | 4.1 | ... | .. | Negative |
| 6 | M | .. | 20 | 54 | 380 | 4.2 | 4.2 | ... | .. | Negative |
| 4 | F | .. | 36 | 60 | 240 | 4.6 | 4.2 | ... | .. | Negative |
| 2 | F | .. | 36 | 59 | 220 | 4.5 | 4.8 | ... | .. | Negative |
| 8 | M | .. | 32 | 50 | 250 | 6.4 | 4.8 | ... | .. | Negative |
| 10 | F | .. | 32 | 50 | 230 | 6.4 | 4.8 | ... | .. | Negative |
| **Group A** | | | | | | | | | | |
| 9 | M | 15 | 12 | 77 | 210 | ... | ... | ... | 19 | Negative |
| 10 | M | 8 | 6 | 86 | 160 | ... | ... | ... | 20 | Negative |
| 3 | F | 22 | 18 | 64 | 210 | ... | ... | ... | 20 | Negative |
| 11 | M | 20 | 18 | 75 | 160 | ... | ... | ... | 27 | Negative |
| 1 | M | 28 | 21 | 53 | 280 | 5.0 | 4.1 | ... | 11 | Negative |
| 2 | M | 30 | 24 | 96 | 180 | 5.6 | 5.3 | ... | 19 | Negative |
| 14 | M | 20 | 18 | 78 | 130 | 8.3 | 6.2 | ... | 32 | Negative |
| 4 | F | 24 | 20 | 100 | 100 | 9.0 | 8.0 | ... | 17 | Negative |
| 6 | M | 15 | 12 | 70 | 190 | ... | ... | ... | 19 | Slight |
| 12 | M | 28 | 26 | 90 | 190 | 5.8 | 5.3 | ... | 29 | Slight |
| 5 | F | 28 | 24 | 75 | 210 | 6.1 | 6.0 | ... | 19 | Moderate |
| 7 | M | 11 | 7 | 103 | 210 | ... | ... | ... | 14 | Severe |
| 8 | M | 18 | 15 | 58 | 210 | ... | ... | ... | 15 | Severe |
| **Group B** | | | | | | | | | | |
| 7 | M | 22 | 18 | 51 | 270 | ... | ... | 1.4 | 3 | Negative |
| 3 | M | 21 | 17 | 53 | 280 | ... | ... | 2.1 | 3 | Negative |
| 4 | M | 8 | 4 | 71 | 150 | ... | ... | 2.2 | 10 | Negative |
| 10 | F | 13 | 11 | 81 | 220 | ... | ... | 2.6 | 5 | Negative |
| 13 | F | 10 | 9 | 58 | 210 | ... | ... | 2.3 | 3 | Negative |
| 2 | M | 25 | 21 | 70 | 310 | 4.2 | 3.7 | 1.4 | 3 | Negative |
| 14 | M | 21 | 19 | 55 | 360 | 5.0 | 5.0 | 2.0 | 4 | Negative |
| 6 | M | 28 | 24 | 59 | 230 | 5.5 | 5.0 | 2.2 | 2 | Negative |
| 1 | M | 26 | 19 | 64 | 280 | ... | ... | 1.3 | 3 | Slight |
| 8 | F | 28 | 24 | 61 | 220 | 6.4 | 5.5 | 1.9 | 4 | Slight |
| 11 | M | 11 | 9 | 75 | 230 | ... | ... | 2.0 | 6 | Moderate |
| 12 | M | 28 | 26 | 84 | 180 | 6.7 | 6.7 | 2.7 | 3 | Moderate |
| **Group D** | | | | | | | | | | |
| 5 | M | 22 | 18 | 90 | 210 | ... | ... | ... | 28 | Negative |
| 13 | F | 27 | 24 | 105 | 230 | 6.1 | 5.2 | ... | 31 | Negative |
| 9 | F | 26 | 19 | 104 | 180 | 4.4 | 5.6 | ... | 16 | Negative |
| 2 | F | 27 | 21 | 100 | 150 | ... | ... | ... | 26 | Slight |
| 10 | M | 20 | 18 | 126 | 190 | ... | ... | ... | 31 | Slight |
| 3 | M | 25 | 21 | 59 | 280 | 5.0 | 3.8 | ... | 25 | Slight |
| 12 | M | 28 | 26 | 113 | 180 | 7.5 | 5.0 | ... | 31 | Slight |
| 8 | F | 27 | 24 | 121 | 230 | 6.5 | 6.5 | ... | 29 | Slight |
| 11 | M | 12 | 10 | 92 | 220 | ... | ... | ... | 13 | Moderate |
| 6 | M | 22 | 18 | 128 | 290 | ... | ... | ... | 26 | Moderate |
| 4 | M | 14 | 10 | 92 | 150 | ... | ... | ... | 39 | Moderate |
| 7 | M | 29 | 25 | 144 | 240 | 8.2 | 5.0 | ... | 29 | Severe |
| **Group E** | | | | | | | | | | |
| 3 | F | 17 | 10 | 47 | 190 | 5.4 | 5.3 | 0.6 | 1 | Negative |
| 5 | F | 13 | 6 | 50 | 170 | ... | ... | 0.5 | 2 | Slight |
| 1 | M | 32 | 24 | .. | 270 | 5.8 | 6.3 | 0.5 | 2 | Slight |
| 4 | F | 32 | 25 | 57 | 280 | 7.5 | 5.0 | 0.7 | 5 | Moderate |
| 2 | M | 12 | 5 | 39 | 190 | 6.3 | ... | 0.5 | 1 | Severe |
| **Group F** | | | | | | | | | | |
| 6 | M | 32 | 28 | 35 | 350 | 4.7 | 3.4 | ... | 0.8 | Negative |
| 3 | M | 33 | 28 | 41 | 350 | 4.6 | 3.6 | ... | 0.9 | Negative |
| 1 | M | 32 | 28 | 50 | 310 | 5.2 | 3.9 | ... | 0.7 | Negative |
| 9 | F | 32 | 28 | 44 | 240 | 6.5 | 5.4 | ... | 0.6 | Negative |
| 5 | M | 33 | 28 | 34 | 370 | 6.0 | 4.3 | ... | 0.8 | Slight |
| 12 | M | 24 | 20 | 31 | 370 | 5.7 | 4.6 | ... | 1.2 | Slight |
| 4 | M | 33 | 28 | 52 | 430 | 6.5 | ... | ... | 0.3 | Moderate |
| 13 | M | 24 | 20 | 47 | 330 | 7.0 | 6.1 | ... | 1.0 | Severe |

TABLE 2.—*Experiment 9*

| Rat | Sex | Duration of Diabetes, Weeks | Duration of Diet 9, Weeks | Terminal Blood Nonprotein N, Mg./100 Cc. | Terminal Protein Excretion, Mg./Day | Terminal Blood Pressure (Indirect), Mm. Hg | Terminal Weight, Gm. | Organ Wt./Body Wt. × 10³: Kidney (Av.) | Organ Wt./Body Wt. × 10³: Heart | Organ Wt./Body Wt. × 10⁴: Adrenal (Av.) | Protamine Zinc Insulin, Units/Day | Urine Sugar, Gm./Week | Evidence of Renal Vascular Disease |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Group C** | | | | | | | | | | | | | |
| 6 | M | .. | 27 | 38 | 2 | 110 | 360 | 4.2 | 3.0 | 0.4 | ... | .. | Negative |
| 4 | M | .. | 27 | 70 | 3 | 150 | 370 | 4.9 | 3.0 | 0.7 | ... | .. | Negative |
| 3 | F | .. | 27 | 60 | 3 | 120 | 240 | 5.8 | 4.0 | 1.1 | ... | .. | Negative |
| 5 | M | .. | 9 | 61 | .. | ... | 240 | 4.8 | 4.6 | 0.9 | ... | .. | Negative |
| 1 | F | .. | 27 | 53 | 0 | 120 | 240 | 5.8 | 4.6 | 1.1 | ... | .. | Negative |
| 2 | F | .. | 27 | 57 | 3 | 105 | 260 | 6.9 | 5.0 | 1.6 | ... | .. | Negative |
| **Group A** | | | | | | | | | | | | | |
| 10 | M | 8 | 5 | 170 | .. | ... | 150 | 7.0 | 4.7 | ... | ... | 25 | Negative |
| 8 | F | 9 | 5 | 103 | .. | ... | 110 | 10.9 | 5.0 | ... | ... | 16 | Negative |
| 3 | M | 24 | 22 | 48 | 23 | 140 | 200 | 7.0 | 5.0 | 1.4 | ... | 18 | Negative |
| 7 | F | 26 | 22 | 52 | 1 | 130 | 210 | 6.7 | 5.2 | 2.0 | ... | 19 | Negative |
| 17 | F | 8 | 4 | .. | 1 | ... | 110 | 6.2 | 5.5 | 2.1 | ... | 11 | Negative |
| 16 | F | 21 | 17 | 73 | 2 | 140 | 150 | 8.4 | 5.7 | 1.6 | ... | 26 | Negative |
| 2 | M | 26 | 21 | 79 | 65 | 125 | 180 | 8.4 | 5.0 | 1.2 | ... | 2 | Slight |
| 14 | M | 26 | 20 | 76 | 41 | 130 | 190 | 7.4 | 5.3 | 0.9 | ... | 14 | Slight |
| 18 | F | 21 | 17 | 45 | 26 | 120 | 190 | 7.1 | 5.4 | 3.5 | ... | 1 | Slight |
| 9 | M | 25 | 22 | 60 | 41 | 110 | 180 | 8.4 | 6.1 | 4.3 | ... | 27 | Slight |
| 12 | M | 23 | 15 | 126 | 80 | ... | 160 | 7.1 | 6.9 | 1.5 | ... | 10 | Severe |
| 15 | M | 26 | 22 | 86 | 83 | 120 | 220 | 7.4 | 7.3 | 1.5 | ... | 19 | Severe |
| 5 | F | 26 | 23 | 194 | 29 | 125 | 160 | 8.0 | 8.8 | 2.3 | ... | 18 | Severe |
| **Group B** | | | | | | | | | | | | | |
| 1 | M | 14 | 7 | 30 | .. | ... | 210 | ... | ... | ... | 2.8 | 3 | Negative |
| 13 | M | 20 | 15 | 51 | 1 | ... | 270 | 5.1 | 3.7 | 1.2 | 3.4 | 4 | Negative |
| 8 | F | 11 | 7 | 69 | 1 | ... | 160 | 5.6 | 4.4 | 2.1 | 3.0 | 3 | Negative |
| 5 | F | 12 | 9 | .. | 1 | ... | 180 | 5.0 | 4.4 | ... | 3.6 | 7 | Negative |
| 10 | M | 24 | 21 | 67 | 7 | 115 | 250 | 5.4 | 4.8 | 1.6 | 3.4 | 4 | Negative |
| 12 | M | 19 | 14 | 73 | 28 | ... | 230 | 5.7 | 5.4 | 1.0 | 3.3 | 3 | Negative |
| 4 | F | 29 | 23 | 61 | 26 | 120 | 230 | 6.3 | 5.7 | 1.8 | 2.6 | 3 | Negative |
| 3 | M | 17 | 11 | 89 | 30 | ... | 220 | 4.8 | 5.9 | 1.2 | 3.0 | 4 | Negative |
| 7 | F | 25 | 21 | 67 | 20 | 110 | 200 | 7.1 | 5.0 | 2.0 | 3.1 | 3 | Slight |
| 11 | M | 16 | 10 | 106 | 17 | ... | 240 | 3.6 | 4.0 | 1.0 | 3.5 | 3 | Moderate |
| 2 | M | 22 | 15 | 247 | 112 | 130 | 250 | 9.6 | 6.0 | 1.4 | 0.6 | 1 | Severe |
| 14 | F | 14 | 11 | 60 | 67 | ... | 190 | 7.2 | 6.3 | 2.7 | 2.0 | 3 | Severe |
| **Group D** | | | | | | | | | | | | | |
| 1 | M | 27 | 20 | 36 | 26 | 125 | 140 | ... | ... | ... | ... | 28 | Negative |
| 4 | F | 18 | 12 | 67 | 28 | ... | 190 | 5.3 | 4.2 | 2.5 | ... | 21 | Negative |
| 10 | M | 29 | 25 | 76 | 37 | 115 | 230 | 6.5 | 4.8 | 1.5 | ... | 20 | Negative |
| 6 | F | 10 | 7 | 80 | 4 | ... | 120 | 5.8 | 5.4 | ... | ... | 23 | Negative |
| 8 | M | 29 | 25 | 85 | 33 | 135 | 190 | 7.0 | 5.5 | 1.9 | ... | 22 | Negative |
| 11 | M | 27 | 22 | 81 | 72 | 150 | 230 | 8.3 | 5.7 | 1.4 | ... | 22 | Negative |
| 3 | M | 27 | 20 | 62 | 4 | 115 | 170 | 10.4 | 5.7 | 1.8 | ... | 20 | Negative |
| 5 | F | 28 | 26 | 60 | 37 | 130 | 190 | 9.8 | 6.3 | 2.0 | ... | 26 | Negative |
| 12 | M | 30 | 25 | 95 | 43 | 135 | 170 | 9.4 | 7.1 | 1.5 | ... | 22 | Slight |
| 9 | M | 20 | 17 | 58 | 17 | ... | 220 | 4.1 | 3.2 | ... | ... | 31 | Moderate |
| 2 | M | 24 | 17 | 92 | 4 | ... | 260 | 8.3 | 7.5 | 1.4 | ... | 24 | Moderate |
| 7 | F | 29 | 25 | 80 | 34 | 120 | 200 | 11.5 | 6.5 | 2.6 | ... | 23 | Severe |
| **Group F** | | | | | | | | | | | | | |
| 7 | F | 24 | 21 | 32 | 24 | 110 | 260 | 6.4 | ... | 1.8 | ... | 0.8 | Negative |
| 8 | F | 24 | 21 | 46 | 9 | 145 | 250 | 6.0 | 4.0 | 1.3 | ... | 0.2 | Negative |
| 9 | F | 24 | 21 | 62 | 11 | 130 | 210 | 7.2 | 4.8 | 1.3 | ... | 0.4 | Negative |
| 3 | M | 28 | 24 | 62 | 6 | 120 | 230 | 7.3 | 5.0 | 1.9 | ... | 0.1 | Negative |
| 1 | M | 32 | 28 | 98 | 31 | 110 | 230 | 8.4 | 4.5 | 0.9 | ... | 0.7 | Slight |
| 4 | F | 23 | 20 | 80 | 20 | 160 | 200 | 6.6 | 6.5 | 2.0 | ... | 0.4 | Moderate |
| 5 | F | 24 | 21 | 63 | 49 | 125 | 220 | 11.4 | 5.5 | 2.1 | ... | 0.8 | Severe |
| 2 | M | 21 | 14 | 98 | 60 | ... | 220 | 7.5 | 7.1 | 1.7 | ... | 0.8 | Severe |
| 10 | F | 24 | 20 | 95 | 72 | 130 | 190 | 9.8 | 7.4 | 2.8 | ... | 3.5 | Severe |
| 6 | F | 24 | 21 | 87 | 66 | 120 | 200 | 12.0 | 8.5 | 2.4 | ... | 1.2 | Severe |

those seen in experiment 5. None of the animals in Group A, 8% in Group B, none in Group C, and 50% in Group D showed evidence of vascular disease.

*Experiment 7* (10%-Sodium Chloride Diet—Kidney-Protected Animals).—In this experiment a number of animals were alloxanized by injecting 50 mg./kg. of alloxan in the form of a 5% aqueous solution into the inferior vena cava. The abdomen was opened under ether anesthesia, clamps were placed on the renal vessels and alloxan was injected. After the injection, the clamps were kept in position for 10 min.; they were then removed, and the abdomen was closed. This procedure led to the development of diabetes while protecting the kidney from the harmful effects of alloxan.[10] Carrasco-Formiguera and Escobar[11] and Jiminez-Diaz, Grande-Covian and de Oya[12] have stated that alloxan diabetes cannot be produced if the renal vessels are clamped. However, if heat is applied to relax the spasm of the visceral vessels, diabetes can be produced readily.[13] It has also been shown by Addis[14] that clamping the renal pedicles for periods up to 90 min. leads to no permanent renal damage. After recovery from the operative procedure, the 11 surviving diabetic animals were placed on the same ad libitum 10%-sodium chloride diet (Diet 6) as those in Experiment 5. No evidence of vascular disease was

TABLE 3.—*Experiment 10*

| Rat | Sex | Duration of Diabetes, Weeks | Duration of Diet 6, Weeks | Duration of Diet 8, Weeks | Terminal Blood Nonprotein N, Mg./100 Cc. | Terminal Blood Pressure (Indirect), Mm. Hg | Terminal Weight, Gm. | Organ Wt./Body Wt. × 10³ Kidney (Av.) | Organ Wt./Body Wt. × 10³ Heart | Urine Sugar, Gm./Week | Evidence of Renal Vascular Disease |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Group D | | | | | | | | | | | |
| 8 | M | 25 | 10 | 11 | 71 | 130 | 310 | 5.5 | 3.2 | 4 | Negative |
| 4 | M | 28 | 6 | 14 | 125 | 145 | 240 | 5.4 | 4.6 | 25 | Negative |
| 5 | F | 21 | 10 | 8 | 72 | 150 | 160 | 7.3 | 5.0 | 21 | Negative |
| 2 | M | 28 | 6 | 14 | 84 | 110 | 300 | 7.6 | 4.6 | 4 | Moderate |
| 14 | M | 27 | 17 | 2 | .. | 140 | 330 | 7.5 | 6.5 | 14 | Moderate |
| 7 | F | 21 | 10 | 7 | 89 | 200 | 180 | 7.5 | 8.3 | 2 | Moderate |
| 12 | M | 31 | 16 | 8 | 69 | 145 | 240 | ... | ... | 18 | Severe |
| 11 | M | 34 | 16 | 11 | 106 | 125 | 220 | 11.3 | 4.8 | 33 | Severe |
| 9 | M | 25 | 13 | 5 | 120 | 220 | 230 | 8.3 | 5.6 | 2 | Severe |
| 10 | M | 26 | 16 | 3 | 118 | 205 | 220 | 6.2 | 8.6 | 2 | Severe |
| 6 | M | 21 | 10 | 8 | 114 | 155 | 130 | 11.9 | 9.3 | 14 | Severe |
| 1 | M | 10 | 6 | 10 | 109 | 180 | 210 | 7.4 | 9.5 | 3 | Severe |
| 3 | M | 21 | 6 | 12 | 92 | 210 | 240 | 7.7 | 11.2 | 2 | Severe |

found in any animals of this group, killed at intervals ranging from five to 46 weeks after alloxanization.

*Experiment 8* (10%-Sodium Chloride Diet—Depancreatized Animals).—Approximately 40 rats were rendered diabetic by subtotal pancreatectomy. As in the previous experiments, the animals were observed for a two-week period to eliminate both those which failed to become permanently diabetic and those which died in the immediate postoperative period. The nine animals which survived were placed on an ad libitum 10%-sodium chloride diet (Diet 6). They maintained a moderately good appearance without the exhibition of insulin and were killed from seven to 22 weeks after depancreatization. None of these animals showed evidence of vascular disease.

*Experiment 9.*—In this experiment about 12 animals in each of five groups, A, B, C, D, and F, were fed a 40%-protein, 10%-sodium chloride diet (Diet 9) for four to 26 weeks, and were

10. Brunschwig, A., and Allen, J. G.: Cancer Res. **4**:45, 1944. Dunn, J. S.; Kirkpatrick, J.; McLetchie, N. G. B., and Telfer, S. V.: J. Path. & Bact. **55**:245, 1943.

11. Carrasco-Formiguera, R., and Escobar, I.: Am. J. Physiol. **152**:609, 1948.

12. Jiminez-Diaz, C.; Grande-Covian, F., and de Oya, J. C.: Nature **158**:589, 1946.

13. Martinez, C.; Gitter, S., and Covian, M. R.: Rev. Soc. argent. biol. **23**:81, 1947, quoted from Diabetes Abstracts **7**:89, 1948.

14. Addis, I.: Glomerular Nephritis; Diagnosis and Treatment, New York, The Macmillan Company, 1949.

killed at intervals varying from eight to 30 weeks after alloxanization. Fifty-four per cent of the animals in Group A, 33% in Group B, none in Group C, 33% in Group D, and 60% in Group F showed evidence of vascular disease (Table 2).

*Experiment 10.*—In this experiment 13 rats were fed a standard diet modified to contain 2%-cholesterol, 10%-sodium chloride (Diet 8). Only diabetic animals on an ad libitum diet (Group D) were studied. Seventy-seven per cent of the animals showed the same typical vascular lesions as were seen in the previous experiments (Table 3).

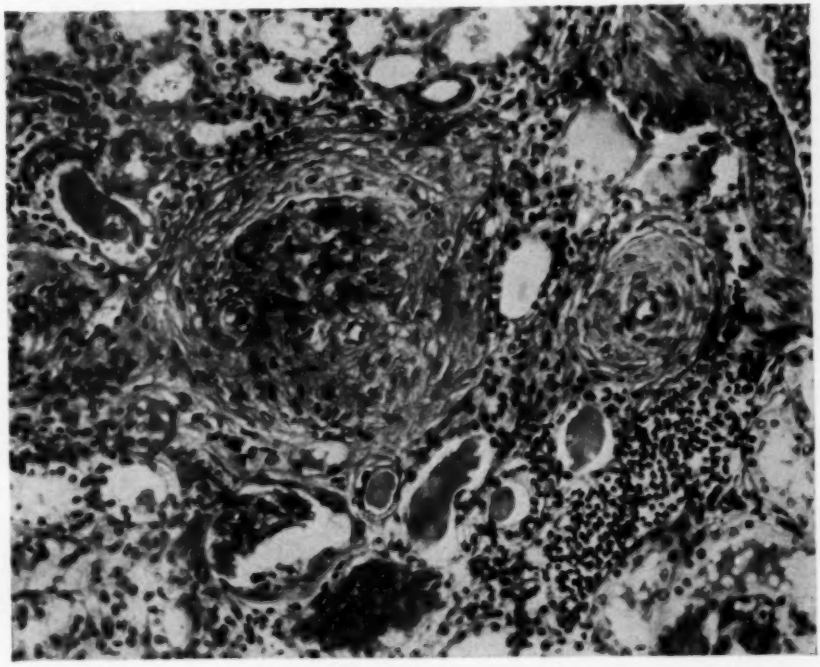

Fig. 1 (kidney—S-3D$^8$; × 200).—Note the extensive proliferation of the vascular coats of the arteriole shown to the right of the fibrosed glomerulus. A moderate amount of inflammatory exudate is also seen in the surrounding tissues.

## PATHOLOGICAL DESCRIPTION

The vascular lesions seen in these animals are characteristic. Macroscopically one may find areas of beading and thickening of the mesenteric and other visible arteries. The microscopic picture is depicted in Figures 1 to 5. The cells of all coats appear swollen, and frequently obvious proliferation of the adventitia and hyalinization of the subendothelial areas are seen. The vascular changes are usually most pronounced in the kidneys and are associated with glomerular fibrosis and tubular degeneration. Vascular lesions may also be seen in other viscera, especially the pancreas, the spleen and the heart. In a few cases in which intracardiac vascular lesions were pronounced, severe degrees of fibrosis have been noted in the heart. No sign of atheromatous change has been encountered.

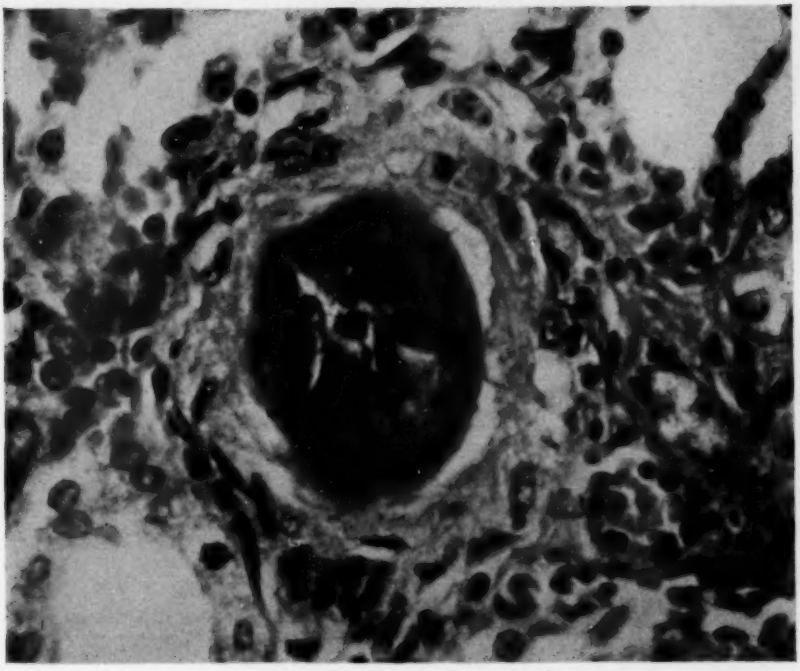

Fig. 2 (kidney—S-7D[7]; × 500).—A marked degree of hyaline degeneration is present in this arteriole with almost complete obliteration of the lumen. There is some edema of the outer layers. Cellular proliferation is minimal or absent.

Fig. 3 (kidney—S-7C[7]; × 500).—This illustrates the appearance of a normal arteriole for comparison with that seen in Figure 2.

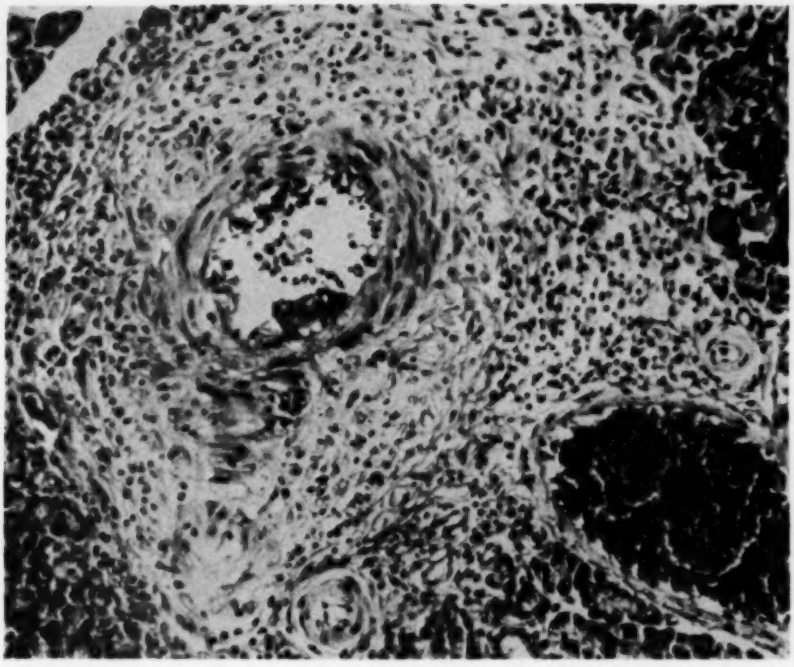

Fig. 4 (pancreas—S-2D$^{s}$; × 200).—This depicts the very extensive perivascular proliferation as well as considerable endothelial proliferation of the vessel wall.

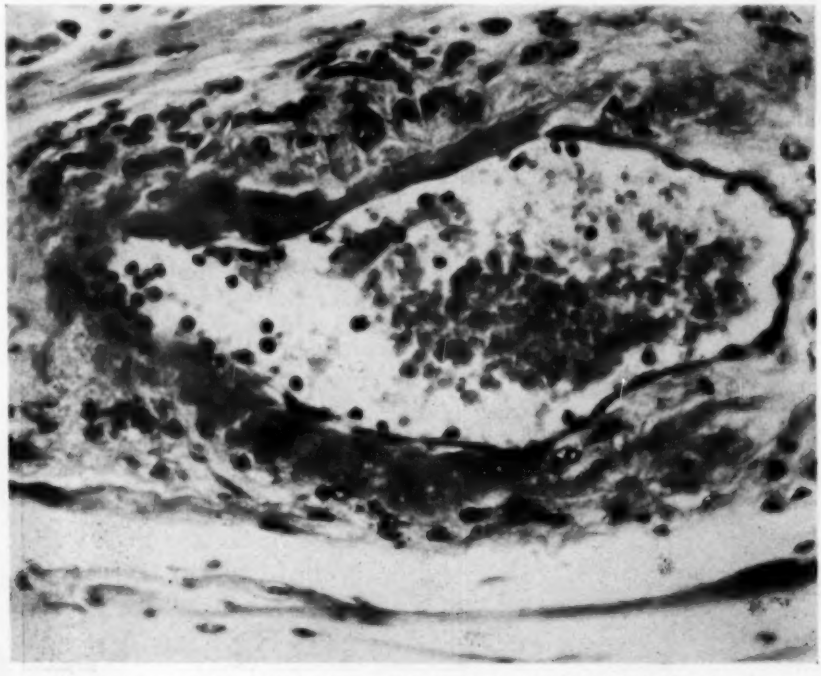

Fig. 5 (heart—S-2D$^{s}$; × 300).—A moderate degree of subendothelial hyalinization is seen with some evidence of edema of the remaining cells of the vascular coat.

## COMMENT

Selye [15] has shown that if sodium chloride is added to the diet of a unilaterally nephrectomized rat exposed to stress, severe vascular disease develops which closely resembles periarteritis nodosa.

Diabetic children in our clinic and at camp have been noted to have an abnormal liking for salt—a taste possibly cultivated during periods of poor control when excessive electrolyte may be lost from the body. It occurred to us that diabetes might act as a stress mechanism and that salt might produce the same result in diabetic rats as in Selye's unilaterally nephrectomized animals.

Although pathological changes were found in the salt-fed diabetic animals (Experiments 5 and 6), the fact that renal vascular lesions appeared in alloxanized, nondiabetic animals (Experiment 5, Group F) led us to believe that diabetes per se was not important. The primary factor appeared to be renal damage due to the nephrotoxic action of alloxan.[10] The observation that vascular lesions did not develop in depancreatized, diabetic animals (Experiment 8) and in diabetic animals whose kidneys were protected from the action of alloxan (Experiment 7) further supports the view that renal damage rather than diabetes is the important factor. Without the excess sodium chloride added to the diet, renal damage from alloxan (Experiments 1 to 4) is insufficient to produce vascular lesions.

The distinct variability in the severity of the vascular lesions can probably be explained by the fact that alloxan may be destroyed in certain animals before it reaches the kidney in toxic concentrations. This may be due to one or more factors: (1) excessive amounts of glutathione or other sulfhydryl compounds which neutralize the effect of alloxan [16]; (2) vasospasm of the renal vessels [11]; (3) poor absorption of alloxan from the site of injection.

Selye [15] has indicated that a high-protein diet potentiates the effect of the administration of sodium chloride, a finding which we were unable to confirm in our present limited series (Experiment 9).

A very high incidence (77%) of vascular change associated with the most severe degrees of renal damage was seen in the group of alloxanized, diabetic animals which were fed 2% cholesterol in addition to the salt diet, although no evidence of atheroma was found.

Wilens and Sproul [17] have shown that periarteritic lesions may arise spontaneously in rats, although they were not encountered prior to the 500th day of life. The lesions produced in our alloxanized, salt-fed groups were not spontaneous in origin, since all animals were sacrificed before the age of 320 days. Furthermore, the nonalloxanized, control group failed to show any evidence of vascular disease.

## SUMMARY

In alloxanized, diabetic rats fed a standard diet (55% carbohydrate) vascular lesions failed to develop during the period of observation. This was also true when the diets were altered to contain 36% fat, 40% protein, or 2% cholesterol.

15. Selye, H.: J. Clin. Endocrinol. **6**:117, 1946; Textbook of Endocrinology, Montreal, Université de Montréal, 1947.

16. Leech, R. S., and Bailey, C. C.: J. Biol. & Chem. **157**:525, 1945.

17. Wilens, S. L., and Sproul, E. E.: Am. J. Path **14**:177, 1938.

In alloxanized, diabetic rats fed a standard diet modified to contain 10% sodium chloride (by weight) severe vascular lesions resembling periarteritis nodosa developed frequently (33 to 80%). A 5%-sodium chloride diet produced similar lesions more slowly and of a less severe nature.

In alloxanized animals that failed to become diabetic, vascular lesions developed when the diet was modified to contain 10% sodium chloride.

Although only a small number of animals were used, a 40%-protein, 10%-sodium chloride diet failed to produce a significant increase in the number of lesions.

The 2%-cholesterol, 10%-sodium chloride diet gave rise to a high incidence (77%) of periarteritic-like lesions, but no atheroma was seen.

Depancreatized, diabetic animals and animals made diabetic by the injection of alloxan while the renal vessels were occluded failed to present vascular lesions.

It is concluded that the vascular lesions are produced by the action of sodium chloride in those cases in which the kidneys have been damaged by alloxan.

## APPENDIX

*Composition of Diets Used in Experiments*

| | Diet 1 | Diet 2 | Diet 3 | Diet 4 | Diet 5 | Diet 6 | Diet 7 | Diet 8 | Diet 9 |
|---|---|---|---|---|---|---|---|---|---|
| Ground whole wheat, % by weight | .... | 61.0 | 25.4 | 49.7 | 50.9 | 55.5 | 58.6 | 54.2 | 42.4 |
| Crude casein, % by weight | .... | 14.2 | 29.7 | 30.1 | 18.9 | 12.9 | 13.65 | 12.6 | 31.1 |
| Whole milk powder, % by weight | .... | 9.5 | 3.9 | 7.7 | 9.2 | 8.6 | 9.1 | 8.4 | 6.6 |
| Wheat germ, % by weight | .... | 5.7 | 2.3 | 4.6 | 5.5 | 5.1 | 5.5 | 5.1 | 3.9 |
| Calcium carbonate, % by weight | .... | 1.4 | 0.6 | 1.1 | 1.4 | 1.3 | 1.25 | 1.3 | 1.0 |
| Alfalfa, % by weight | .... | 1.0 | 0.4 | 0.85 | 1.0 | 0.9 | 0.95 | 0.9 | 0.7 |
| Butter (salt-free), % by weight | .... | 6.2 | 2.6 | 5.1 | 6.1 | 5.7 | 5.95 | 5.5 | 4.3 |
| Sodium chloride, % by weight | .... | 1.0 | 0.4 | 0.85 | 1.0 | 10.0 | 5.0 | 10.0 | 10.0 |
| Cholesterol, % by weight | .... | .... | .... | .... | 2.0 | .... | .... | 2.0 | .... |
| Hydrogenated cottonseed oil (crisco®), % by weight | .... | .... | 30.9 | .... | .... | .... | .... | .... | .... |
| Salt mixture 185, % by weight | .... | .... | 3.8 | .... | .... | .... | .... | .... | .... |
| Vitamin supplement, % by weight | .... | .... | .... | 0.005 | .... | .... | .... | .... | .... |
| Fox chow, % by weight | 100 | .... | .... | .... | .... | .... | .... | .... | .... |
| Carbohydrate, % by weight | 49.9 | 55.0 | 24.0 | 45.0 | 54.0 | 50.0 | 52.8 | 48.8 | 38.2 |
| Fat, % by weight | 5.4 | 10.0 | 36.0 | 8.0 | 10.0 | 9.0 | 9.6 | 8.9 | 7.0 |
| Protein, % by weight | 23.9 | 27.0 | 37.0 | 40.0 | 26.0 | 25.0 | 25.9 | 24.0 | 40.0 |
| Sodium chloride, % by weight | 1.3 | 1.0 | 0.4 | 0.85 | 1.0 | 10.0 | 5.0 | 10.0 | 10.0 |
| Cholesterol, % by weight | .... | .... | .... | .... | 2.0 | .... | .... | 2.0 | .... |
| Roughage, % by weight | 10.5 | 7.0 | 2.6 | 6.15 | 7.0 | 6.0 | 6.7 | 6.3 | 4.8 |

# LIPEMIA OF RABBITS FOLLOWING UNILATERAL OCCLUSION OF THE RENAL VESSELS

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ISCHEMIA has been used frequently to injure a kidney experimentally. No detailed studies have been made of the effect of this procedure on the serum lipids. Since certain forms of renal damage are associated with changes in the lipid content of the serum, renal ischemia also may be accompanied by similar variations in the lipids.

## MATERIAL AND METHODS

The serum lipids and the nonprotein nitrogen of the blood of rabbits were determined preceding, and at one to three-day intervals following, unilateral occlusion of the renal artery or vein. In seven rabbits the renal vein was clamped 12 to 20 min.; in five the renal vein was ligated but not divided; in one the renal artery was ligated, and in one the renal artery and vein were ligated. The rabbits were anesthetized with diallylbarbituric acid, 0.7 cc., or pentobarbital sodium, 25 mg. per kilogram.

As control of the surgical procedures, the effects of 20-min. occlusion and permanent ligation of the splenic vein, surgical exposure of one kidney, and anesthesia alone were tested. Blood was removed from the marginal ear vein and the lipids were determined at the same time as in the case of the animals with renal ischemia. Significant variations did not occur in the lipid content of the serum of the control animals.

Blood chemical analysis was performed according to the methods described.[1] Animals were fed a balanced ration during the experiment. Food was not removed from the cage prior to withdrawing blood for chemical analysis.

## RESULTS

Renal ischemia was produced in a total of 14 rabbits. During one to three days after the renal vessels had been ligated or clamped the total serum esterified fatty acid was significantly elevated in all but one (table). The fatty-acid concentration

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From the Henry Baird Favill Laboratory, St. Luke's Hospital, and the Department of Pathology, University of Chicago.

Dr. Johnson was the John Jay Borland Fellow, St. Luke's Hospital. He is now with the Armed Forces.

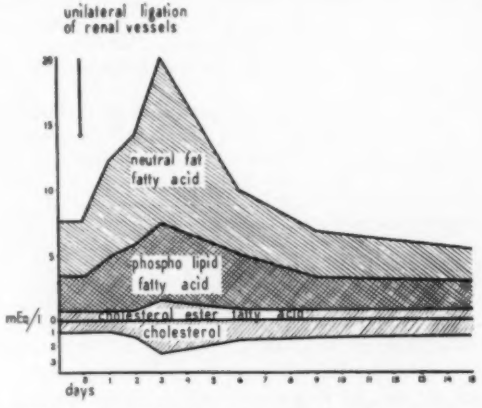
Miss Carbonaro is the Seymour Coman Fellow in the Department of Pathology, University of Chicago.

1. Bauer, F. C., Jr.; Johnson, G. C.; Carbonaro, L., and Hirsch, E. F.: Changes in the Serum Lipids of Rabbits with Acute Uranyl Nitrate Poisoning, A. M. A. Arch. Path. **51**:441 (April) 1951.

gradually diminished and approached the control level about one to two weeks later. Changes in the various serum lipid fractions following unilateral ligation of the renal artery and vein in Rabbit XX10 are plotted in the chart. The maximum increase is in the neutral-fat fraction.

*Changes in Serum Esterified-Fatty-Acid Concentration Following Unilateral Renal Ischemia*

| Rabbit | Control Serum Esterified-Fatty-Acid Concentration mEq./L. | Surgical Procedure | Serum Esterified-Fatty-Acid Concentration 1 to 3 Days After Surgical Procedure, mEq./L. |
|---|---|---|---|
| XX1 | 4.7 | 20-min. renal-vein compression | 21.2 |
| XX6 | 4.7 | 12-min. renal-vein compression | 38.1 |
| XX7 | 5.6 | 12-min. renal-vein compression | 11.4 |
| XX | 4.2 | 15-min. renal-vein compression | 19.7 |
| XX9 | 5.8 | 15-min. renal-vein compression | 20.5 |
| XX12 | 4.4 | 12-min. renal-vein compression | 12.2 |
| B4-3 | 6.7 | 15-min. renal-vein compression | 11.4 |
| XX8 | 5.6 | Unilateral renal-vein ligation | 46.2 |
| UN4 | 9.6 | Unilateral renal-vein ligation | 54.2 |
| UN6 | 4.4 | Unilateral renal-vein ligation | 24.3 |
| UN7 | 6.7 | Unilateral renal-vein ligation | 72.7 |
| UN10 | 4.2 | Unilateral renal-vein ligation | 8.8 |
| UN8 | 7.3 | Unilateral renal-artery ligation | 12.5 |
| XX10 | 7.6 | Unilateral renal-artery and renal-vein ligation | 20.0 |




Changes in serum lipid fractions following ligation of the renal artery and vein in Rabbit XX10.

The blood nonprotein nitrogen increased to about twice the control level at about the same time that the fatty-acid level reached its peak and then gradually returned to or near the control level.

Rabbit XX8 was killed three days after the left renal vein had been ligated and when the serum fatty acids had reached 46.2 mEq. per liter. The left kidney was enlarged and mottled with dark red and pale infarcts, and only a small amount of normal tissue remained. Histologically, large regions were necrotic, and only out-

lines of renal structures remained; the other tissues were hemorrhagic. Small portions of kidney tissue escaped necrosis. Tissues of the opposite kidney revealed no significant changes except the presence of granular precipitates in the lumens of the tubules. The spleen had deposits of coarsely granular iron pigment. In sections of tissues fixed in formaldehyde solution U. S. P. and stained with Sudan IV for fat, scattered cells lining the tubules contained many fat droplets. The cells of the liver had only a few fat droplets, but some of the myocardial fibers had many.

Rabbit UN6 was killed when the serum lipids had returned to near the control level and 30 days after ligation of the renal vein. The kidney with the ligated vein had scars under the capsule. The deeper levels of the cortex and the medulla and sections from the opposite kidney were without significant changes. A few tubules were dilated, and some had hyaline casts. In sections stained with Sudan IV the lining cells of a few tubules contained some large droplets of fat. There were no appreciable fatty changes of the liver.

Rabbit UN7 was killed 50 days after ligation of the renal vein, when the serum lipid had returned to near the control level. The kidney with the ligated vein was greatly reduced in size, was firm, gray, and mottled with tan-brown regions. In sections fixed in Zenker's solution and stained with hematoxylin and eosin, much of the renal parenchyma was necrotic or replaced by scar tissue. Many of the tubules contained basophilic precipitates like calcium, and often no cellular detail remained. Foreign-body giant cells were associated with some of the granular precipitates. There were a few aggregates of large cells with abundant vacuolated cytoplasm like foam cells. The necrotic regions had deposits of lipid in sections stained with Sudan IV.

## COMMENT

Ischemia was used to produce experimental renal injury in rabbits by Wartman, Rusterholz, and Tucker [2] and Badenoch and Darmady.[3] In their experiments, histologic changes were confined principally to the renal tubules. Studies of blood lipids were not made.

Variations in the effect of renal ischemia have been caused by incomplete occlusion or damage of the renal vessel by the clamp and spontaneous spasm of the artery after the clamp was removed.[3] Stoll and Carlson [4] clamped the renal artery or vein of dogs for one to 20 min. and found that anuria of five minutes' to three hours' duration resulted after the clamp was removed. They explained the postanemic changes of the kidney on the basis of vascular spasm. Rowntree, Fitz, and Geraghty [5] observed extensive collateral circulation after clamping a renal vein in dogs. These circulatory factors probably caused some variations in the results of our experiments.

Fishberg [6] described a nephrotic syndrome in a patient with thrombosis of the inferior vena cava. Anasarca, proteinuria, hypoproteinemia, and lipemia occurred, and necropsy disclosed canalized thrombi of the inferior vena cava and renal veins.

2. Wartman, W. B.; Rusterholz, A. P., and Tucker, J. M.: Am. J. Path. **26**:235, 1950.

3. Badenoch, A. W., and Darmady, E. M.: J. Path. & Bact. **59**:79, 1947.

4. Stoll, J. E., and Carlson, A. J.: Am. J. Physiol. **67**:153, 1923-1924.

5. Rowntree, L. G.; Fitz, R., and Geraghty, J. T.: The Effects of Experimental Chronic Passive Congestion of Renal Function, Arch. Int. Med. **11**:121 (Feb.) 1913.

6. Fishberg, A. M.: Hypertension and Nephritis, ed. 4, Philadelphia, Lea & Febiger, 1939, p. 374.

Derow, Schlesinger, and Savitz [7] reported the occurrence of the nephrotic syndrome in a patient with occlusion of the inferior vena cava and of the renal and portal veins. The total cholesterol of the blood rose from 164 mg. per 100 cc. to as high as 926 mg. per 100 cc. after nine months. Microscopic examination of the kidneys revealed the glomeruli to be unchanged. There was cloudy swelling of the tubular epithelium, and in the lumens of many there was granular debris. The interstitial tissue was edematous and had an occasional focus of round cells.

## SUMMARY

Unilateral ligation of the renal artery or vein or clamping the renal vein of rabbits for 12 to 20 min. produced a transient lipemia of about one to two weeks' duration. All lipids participated in this increase, but the greatest increase was in the neutral-fat fraction.

7. Derow, H. A.; Schlesinger, M. J., and Savitz, H. A.: Chronic Progressive Occlusion of the Inferior Vena Cava and the Renal and Portal Veins, with the Clinical Picture of the Nephrotic Syndrome: Report of a Case, with a Review of the Literature, Arch. Int. Med. 63:626 (April) 1939.

# EFFECT OF CORTISONE ON THE ARTHUS PHENOMENON AS RELATED TO THE TIME OF SENSITIZATION

## A Comparison of Histopathologic Changes

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THE RESULTS of experimental studies of the effects of cortisone and other cortical steroids on hypersensitivity and antibody formation are remarkably inconsistent. It was thought that animals treated with cortisone during the period of active sensitization might react differently from those treated with cortisone subsequent to sensitization, and that this might explain some of the apparent inconsistencies. Therefore, the object of these experiments was to compare the effects produced by cortisone when it is administered during the period of active sensitization with those when it is administered immediately after this period.

The Arthus phenomenon was selected as a good example of experimental hypersensitivity. It is a localized antigen-antibody reaction accompanied by acute inflammation and vascular necrosis.[1] According to some authors,[2] the tissue damage is due to impairment of nutrition secondary to vascular damage and clogging of tissue spaces with exudate and hemorrhage. Moreover, there is a definite relationship between the severity of reaction and the precipitin titer of the serum. In man, this type of reaction is thought to be the basic one in diseases of altered reactivity.[3] Experimentally, the reaction can easily be produced in the rabbit.

### METHODS AND MATERIALS

Twenty-four young adult female albino rabbits were divided into three groups of eight animals each. Group 1, the control, was actively sensitized with horse serum. A dose of 1.25 mg. of protein per kilogram was given intravenously five times per week for two weeks; then 2.5 mg. per kilogram was given five times for one week. On the fifth day after the last intravenous injection, an intracutaneous shocking dose of 0.1 cc. of horse serum (3 mg. of protein per cubic centimeter) was given. Group 2 was similarly sensitized and at the same time given cortisone acetate intramuscularly in doses of 2 mg. per kilogram per day. The cortisone treatment was started on the first day of sensitization and discontinued on the fifth day after sensitization. A shocking intracutaneous injection was also given on the fifth day as in Group 1. Group 3 was similarly sensitized, but cortisone was given only on each of the five days following the last intravenous injection. A similar intracutaneous shocking dose of horse serum was also given on the fifth day.

After the intracutaneous shocking dose was given, 5 cc. of blood was obtained from each animal. Antibody titers were run on the pooled serums from each of the three groups as

From the Institute of Pathology, Western Reserve University, and University Hospitals of Cleveland.

1. Opie, E. L.: J. Immunol. **9**:259, 1924.
2. Cannon, P. R., and Marshall, C. E.: J. Immunol. **40**:127, 1941. Rich, A. R., and Follis, R. H.: Bull. Johns Hopkins Hosp. **66**:106, 1940.
3. Rich, A. R., and Gregory, J. E.: Bull. Johns Hopkins Hosp. **73**:239, 1943; **72**:65, 1943.

follows: Duplicate dilutions of normal horse serum were made up for each pool of serums in 0.9% saline solution, starting with 1:20 dilution. Three sets of 11 tubes each were thus used, the eleventh tube serving as a control. To the control tubes, 0.5 cc. of 0.9% saline solution was added instead of diluted normal horse serum. To each tube in Row A (see table) 0.5 cc. of undiluted antihorse rabbit serum of Pool 1 (serums of Group 1) was added; to Row B, 0.5 cc. of Pool 2; to Row C, 0.5 cc. of Pool 3 was added. The tubes were incubated for one hour at 37 C. and the first reading recorded. After the tubes were kept overnight in an icebox a second reading was recorded.

The skin lesions of each animal were examined 24 and 48 hr. after the shocking intradermal injection, and interpreted as follows [4]:

4+ 10 mm. confluent hemorrhage and edema—persists 48 hr.

3+ 15 mm. petechiae and edema—persists 48 hr.

2+ 10-15 mm. petechiae and edema—persists 48 hr.

1+ 5-10 mm. petechiae and slight edema—persists 48 hr.

± less than 5 mm. petechiae—disappears after 24 hr.

0 no reaction

*Precipitation of Normal Horse Serum by Antihorse Rabbit Serum* *

| Sample No. | Normal Horse Serum | +0.5 Cc. Antihorse Rabbit Serum | | | | | |
|---|---|---|---|---|---|---|---|
| | | A Pool 1 | | B Pool 2 | | C Pool 3 | |
| 1 | 1: 20-0.5 cc. | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ |
| 2 | 1: 40 | 4+ | 4+ | 2+ | 2+ | 4+ | 4+ |
| 3 | 1: 80 | 4+ | 4+ | 2+ | 2+ | 4+ | 4+ |
| 4 | 1: 160 | 4+ | 4+ | ± | + | 4+ | 4+ |
| 5 | 1: 320 | 3+ | 3+ | ... | ... | 4+ | 4+ |
| 6 | 1: 640 | 3+ | 3+ | ... | ... | 3+ | 3+ |
| 7 | 1: 1,280 | 2+ | 2+ | ... | ... | 2+ | 2+ |
| 8 | 1: 2,560 | ± | ± | ... | ... | + | + |
| 9 | 1: 5,120 | ... | ... | ... | ... | ... | ... |
| 10 | 1:10,240 | ... | ... | ... | ... | ... | ... |
| 11 | 0 | ... | ... | ... | ... | ... | ... |

* The preparations were kept for one hour at 37 C. and overnight in the icebox.

The animals were then sacrificed, and sections of skin lesions, gonads, uterus, lymph nodes, thymus, and adrenal were fixed in formaldehyde solution, sectioned, and stained with hematoxylin and eosin. A lipid stain (Sudan III) was done on the adrenal. Sections of pituitary and bone marrow were fixed in Carnoy's solution. The bone marrow was stained with hematoxylin and eosin and the Kingsley [5a] stain. The pituitary was stained for granules by the Pearse method.[5b]

The 24 animals used in this experiment were thoroughly acclimated to the laboratory for three months before injections were begun. In none did diarrhea develop, nor did any lose more than 10% of its original weight.

## RESULTS

The results of the precipitation of normal horse serum by antihorse rabbit serum were as follows (also shown in the table): In Pool 2, the serums of rabbits treated daily with cortisone, precipitation of horse serum was completely absent after dilution 1:320. In Pools 1 and 3, the serums of the controls and the five-day cortisone-treated rabbits, respectively, precipitation occurred in dilutions up to and including 1:2,560.

4. Benacerraf, B., and Kabat, E. A.: J. Immunol. **64**:1, 1950.

5. (*a*) Kingsley, D. M.: Stain Technol. **10**:127, 1935. (*b*) Pearse, A. G. E.: Ibid. **25**:95, 1950.

Examination of the Arthus skin lesions showed a 4+ reaction in seven of eight animals. The skin of the other animal showed a 1+ reaction. Microscopically, the 4+ lesions showed a dense infiltrate of heterophils,[6] lymphocytes, and monocytes involving the corium and subcutaneous fat (Fig. 1*A*). There was a characteristic focal acute necrotizing inflammation of arterioles, capillaries, and venules in and around the infiltrate (Fig. 2*A*). The 1+ lesion showed considerably less infiltrate and no vascular necrosis. The skin lesions of the animals of Group 2 all showed a 1+ reaction. Microscopically, there was a scattered infiltrate of heterophils, lymphocytes, and monocytes in the corium and superficial subcutaneous fat (Fig. 1*B*). Vascular necrosis was not identified in any of these sections (Fig. 2*B*). The skin lesions of the animals of Group 3 were intermediate in severity. One animal showed a 3+ reaction, four showed a 2+ reaction, and three showed a 1+ reaction. Although microscopically there were edema and hemorrhage in the corium corresponding to the gross appearance, the cellular infiltrates in all were moderately reduced (Fig. 1*C*) and in this respect similar to those of Group 2. However, vascular necrosis was present (Fig. 2*C*) in all but two.

The lymph nodes (Fig. 3*A*) and thymuses of animals of Group 1 were normal. Those of Group 2 showed moderate atrophy, evidenced by decreased numbers of lymphocytes (Fig. 3*B*) and dilated sinuses in the lymph nodes. The lymphatic tissues of animals of Group 3 (Fig. 3*C*) were similar to those of Group 2.

The bone marrows of animals of Group 2 were normal (Fig. 3*D*). They were composed of approximately 80% cells and 20% fat. Megakaryocytes were of adequate number. The bone marrows of Group 2 were 50% cells and 50% fat (Fig. 3*E*). Normal proportions of cells were present, however, and megakaryocytes were adequate. The marrows of Group 3 (Fig. 3*F*) animals were similar to those of Group 1.

The adrenals of animals of Group 1 were normal (Fig. 4*A*). There were moderate amounts of fat in the zona fasciculata and the zona reticularis and slight amounts throughout the zona glomerulosa. The adrenals of Group 2 showed moderate cortical atrophy. The compact ovoid groups of cells in the glomerulosa were replaced by loosely arranged tubular formations[7] (Fig. 4*B*). Occasionally, there was slight pyknosis of cells, but no inflammatory infiltrate was observed. In the superficial one-fourth of the fasciculata[8] the cords of cells lay slightly apart. Fat was minimal in the glomerulosa, slightly decreased in the superficial fasciculata, normal elsewhere. The adrenals of Group 3 (Fig. 4*C*) showed changes similar to Group 2 but much less pronounced. Fat content and distribution were normal.

The pituitaries of Group 1 animals were normal.[9] The alpha cells, constituting an estimated 40% of the total cells and located throughout the anterior lobe, contained tightly packed fine yellow granules. The beta cells, 11% of the total number of cells, and lying mostly around the periphery of the anterior lobe, were slightly

6. Wintrobe, M. M.: Clinical Hematology, Philadelphia, Lea & Febiger, 1946. Downey, H.: Handbook of Hematology, New York, Paul B. Hoeber, Inc., 1938, vol. 2.

7. White, A.: Bull. New York Acad. Med. **24**:26, 1948.

8. Greep, R. O., and Deane, H. W.: Ann. New York Acad. Sc. **50**:596, 1950.

9. Maximow, A. A., and Bloom, W.: A Text-Book of Histology, Philadelphia and London, W. B. Saunders Company, 1949. Friedgood, H. B., and Dawson, A. B.: Anat. Rec. suppl. 3 **67**:18, 1937. Herring, P. T.: Quart. J. Exper. Physiol. **1**:121, 1908.

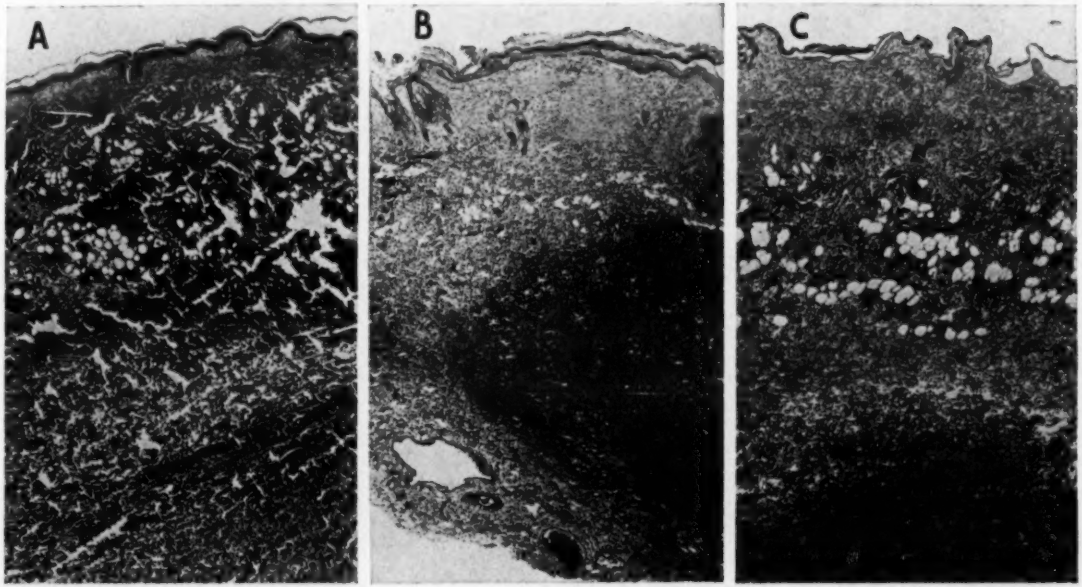


Fig. 1.—*A*, skin of Group 1 animal showing diffuse exudate of Arthus reaction. × 20. *B*, skin of Group 2 animal treated with cortisone during sensitization. Note localized exudate. × 20. *C*, skin of Group 3 animal treated with cortisone after sensitization. Note partially localized and reduced amount of exudate. × 20.

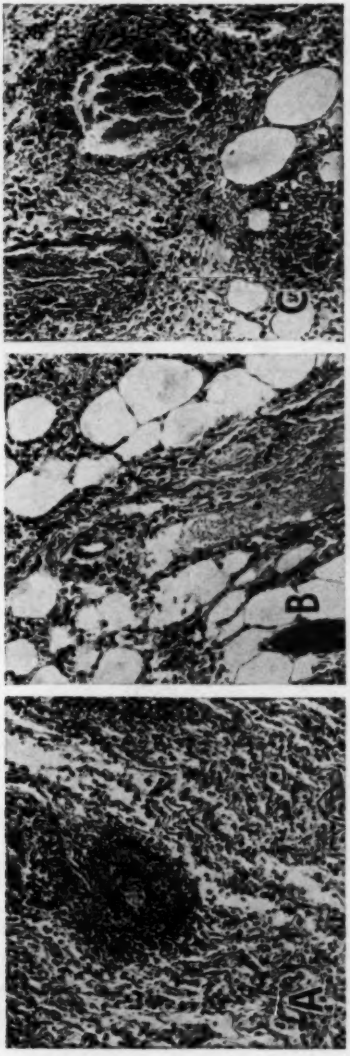


Fig. 2.—*A*, skin of Group 1 animal showing acute necrotizing arteriolitis in corium at site of Arthus reaction. × 150. *B*, skin of Group 2 animal showing normal blood vessels in corium at site of Arthus reaction. × 150. *C*, skin of Group 3 animal showing acute necrotizing inflammation of arteriole and venules at site of Arthus reaction. × 150.

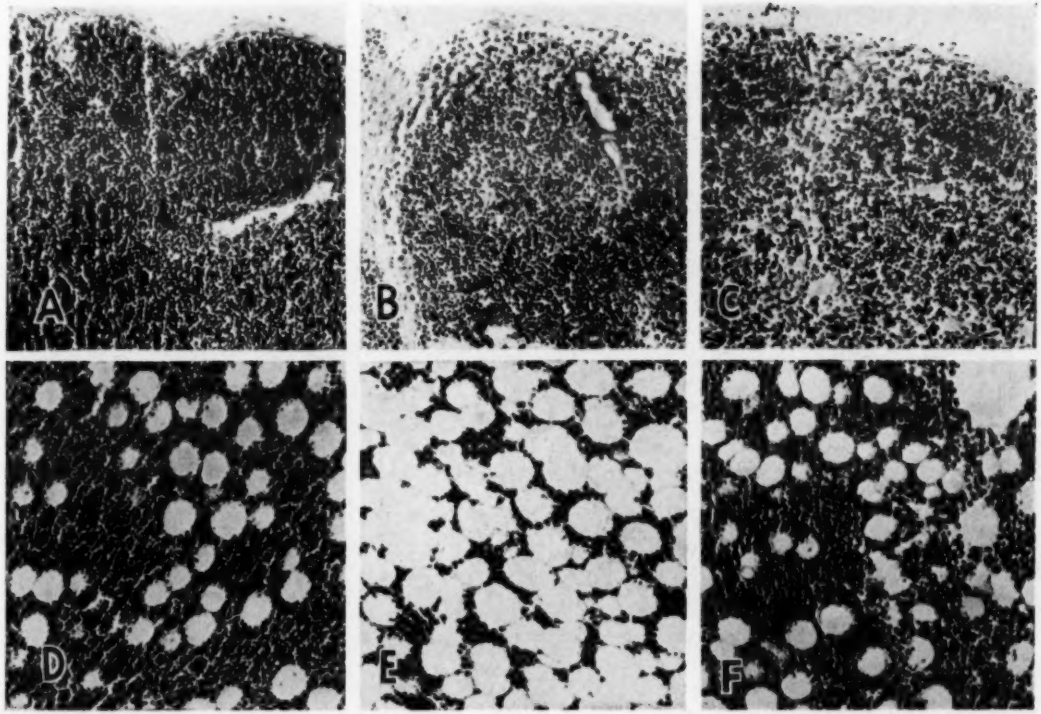


Fig. 3.—*A*, normal lymph node of Group 1 animal. × 150. *B*, moderate atrophy of lymph node of Group 2 animal treated with cortisone during sensitization. × 150. *C*, moderate atrophy of lymph node of Group 3 animal treated with cortisone after sensitization. × 150. *D*, normal bone marrow of Group 1 animal. × 150. *E*, moderate atrophy of bone marrow of Group 2 animal. × 150. *F*, normal bone marrow of Group 3 animal. × 150.

Fig. 4.—*A*, normal adrenal cortex of Group 1 animal. × 150. *B*, peripheral adrenal cortex of Group 2 animal showing degeneration of cells and tubular formation. × 150. *C*, peripheral adrenal cortex of Group 3 animal. Note beginning degeneration and slight tubular formation in zona glomerulosa. × 150.

larger than the alpha cells and had large, pink, loosely packed granules. The remainder of the cells were of the chromophobe type. In Group 2 the beta-cell granules were moderately reduced in number and the cytoplasmic outlines of the individual cells were poorly defined. The alpha cells and the chromophobes were not altered. The pituitaries of Group 3 showed no significant change.

The ovaries and uteri of the three groups showed no characteristic change.

## COMMENT

The depression of antibody formation and inhibition of vascular necrosis by cortisone in the Arthus type reaction have been previously reported by several investigators [10] who worked with rabbits. They also treated the animals with cortisone during the period of sensitization. The inhibition of exudate formation or antiphlogistic effect of cortisone has been demonstrated in mice.[11]

There are many apparent contradictions concerning the action of cortisone on antibody formation and the Arthus reaction. Some investigators,[12] working with rabbits and mice, have asserted that adrenal cortical hormones enchance circulating-antibody titer. The elevations following a single injection of cortisone in a previously sensitized animal were temporary and were probably a nonspecific anamnestic response. Fischel, Le May and Kabat [13] failed to demonstrate the reaction in rabbits three months after cessation of immunization.

Other investigators have found that adrenal cortical hormones have no effect on antibody formation. One group [14] showed that adrenalectomized cats maintained on desoxycorticosterone acetate produced antibodies to levels comparable with those found for intact controls. Single large doses of the steroid did not alter the response. Mirick [15] has reported that 12 patients, already receiving cortisone or adrenocorticotropin (ACTH) for lupus or asthma and then vaccinated against Type 2 pneumococcus, produced antibody as promptly and in as high a titer as the controls. There was, however, a depression of induced skin sensitivity in some patients with a persistent drop in the gamma-globulin serum fraction in many. A final group [16] has reported that cortisone (34 mg. daily) given to rabbits previously sensitized did not exert any influence on the appearance of the Arthus reaction.

Some of these divergent results can possibly be explained by differences of technique, dosage, and animal used, but the most important consideration is whether cortisone was administered during or after the sensitization period. As demon-

10. Germuth, F. G., and Ottinger, B.: Proc. Soc. Exper. Biol. & Med. **74**:815, 1950. Germuth, F. G.; Nedzel, G. A.; Ottinger, B., and Oyama, J.: Ibid. **76**:177, 1951. Seifter, J.; Ehrich, W. E.; Begany, A. J., and Warren, G. H.: Ibid. **75**:337, 1950. Rich, A. R.; Berthrong, M., and Bennett, I. L., Jr.: Bull. Johns Hopkins Hosp. **87**:549, 1950

11. Dougherty, T. F., and Schneebeli, G. L.: Proc. Soc. Exper. Biol. & Med. **75**:854, 1950.

12. Dougherty, T. F.; Chase, J. H., and White, A.: Proc. Soc. Exper. Biol. & Med. **58**:135, 1945. Eisen, H. N.; Mayer, M. M.; Moore, D. H.; Tarr, R. R., and Stoerk, H. C.: Ibid. **65**:301, 1947. Hammond, C. W., and Novak, M.: Ibid. **74**:155, 1950.

13. Fischel, E. E.; Le May, M., and Kabat, E. A.: J. Immunol. **61**:89, 1949.

14. Thatcher, J. S.; Houghton, B. C., and Ziegler, C. H.: Endocrinology **43**:440, 1948.

15. Mirick, G. S.: J. Clin. Invest. **29**:836, 1950.

16. Harris, S., and Harris, T. N.: Proc. Soc. Exper. Biol. & Med. **74**:186, 1950.

strated by the results of this experiment, the time relationship between the administration of cortisone and the sensitization of the animal is an extremely critical one, and the results depend directly on this factor.

The mechanism of cortisone action is not known. It seems to be that of a nonspecific depressant capable of modifying any given step of the immune response if it is present at the time that that particular step is being initiated.

Although lymphoid atrophy was moderate in the two treated groups of animals, the difference was not considered significant; hence no relationship to decreased antibody titer was demonstrated.

The reported histologic alterations produced by cortisone in tissues of other animals vary from those seen here. In rats,[17] daily subcutaneous doses of cortisone acetate (3 mg.) produced marked atrophy of the inner zones of the adrenal cortex while the zona glomerulosa remained intact. These changes became apparent after the 10th day. Laqueur [18] has also reported that in the pituitaries of five of eight patients the granules of the beta cells are replaced by lumpy masses of hyaline basophilic material as early as five days after the onset of treatment.

## SUMMARY

When cortisone is given to rabbits during the time of active immunization, serum-antibody formation is decreased. The severity of the Arthus reaction, said to be directly related to serum-precipitin titer, is likewise decreased. Histologically, at the injection site there is a small exudate and no vascular necrosis. When cortisone is given to rabbits after the completion of sensitization, little or no effect is produced on the serum titer or the vascular lesions of the Arthus reaction; however, the amount of exudate is reduced. The dosage of cortisone used, 2 mg. of the acetate per kilogram per 24 hr., is less than two times the therapeutic dose used in man.

Cortisone given over a period of three weeks produces definite but nonspecific histologic changes. Bone marrow, lymph nodes, thymus, and adrenals show moderate atrophy. In the adrenals the atrophy is confined to the zona glomerulosa and the peripheral zona fasciculata, where there is an associated decrease in fat. In the pituitary the granules of the beta cells are decreased.

When cortisone is given for a period of five days, there are minimal atrophy of the adrenal cortex and slight to moderate atrophy of the thymus and the lymph nodes.

## CONCLUSIONS

Cortisone given to rabbits during active sensitization decreases antibody formation, reduces the amount of exudate, and prevents the vascular necrosis of the Arthus reaction.

Cortisone given to rabbits after sensitization does not affect antibody titer or the vascular necrosis of the Arthus reaction but does reduce the amount of exudate.

Short courses of cortisone produce slight atrophy of lymphoid tissue, whereas longer courses produce atrophy of bone marrow, atrophy of the peripheral cortex of the adrenal, and decrease of the granules in the beta cells of the pituitary.

Dr. Louis Pillemer and Mrs. Livia Blum performed the serum-antibody determinations.

17. Stebbins, R. B.: Federation Proc. **9**:345, 1950.
18. Laqueur, G. L.: Science **112**:429, 1950.

# LOCALIZATION OF THROMBOSIS IN THE MAIN ARTERIES AND DEEP VEINS OF THE LOWER LIMB

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DURING an investigation of arterial thrombosis in the lower limb, based on autopsy material (Lindbom,[1] 1950), the venous thrombi in the concomitant veins were also recorded in a part of the series. It was intended to study the localization of deep venous thrombosis and to find out whether any relationship exists between the localizations of arterial and venous thrombosis in the main vessels of the lower limb.

From the works of Rössle,[2] Neumann,[3] and Frykholm [4] it has been established that in the majority of cases venous thrombosis has its origin in the muscular veins of the calf. Arterial thrombosis in the lower limb was recently studied by me. The posterior tibial was found to be the most frequently and the peroneal the least frequently thrombosed of the main arteries of the lower limb. The distal half of the posterior tibial artery was more often thrombosed than the proximal half.

## METHOD

Arteriography of the lower limb was performed on 186 cadavers. The main arteries containing the contrast medium were dissected down to the ankle together with their concomitant veins adherent to the arteries. The vessels of the foot were dissected in a few cases only. On the fresh specimens the extent of venous thrombosis could easily be judged. In a part of the series the lengths of the venous thrombi were marked by means of pins, and roentgenograms of the specimens were made. On these roentgenograms both arterial and venous thromboses were thus recorded. Thrombi of both kinds were registered on a schematic drawing, one card for every leg. Histograms were then made. A detailed account of these proceedings was given in the work of Lindbom in 1950.

## MATERIAL

Mainly cadavers of more than 50 yr. of age were investigated. The veins were studied during the last part of the investigation only. In 22 limbs both arterial and venous thrombi were found. Venous thrombi alone were found in 14 limbs. There was a certain overproportion of arteriosclerotic cases, but no other selection was made.

## RESULTS

The localization of arterial thrombosis in the 22 limbs (Fig. 1*B*) was the same as in a larger material which was described in the already mentioned work of Lindbom.[1] The localization of venous thrombosis was in accordance with the

From the Roentgendiagnostic Department of Karolinska Sjukhuset, Stockholm.

1. Lindbom, Å.: Acta radiol, supp. 80, 1950.
2. Rössle: Arch. path. Anat. **300**:180, 1937.
3. Neumann, R.: Arch. path. Anat. **301**:708, 1938.
4. Frykholm, R.: Surg., Gynec. & Obst. **71**:307, 1940.

view of earlier authors. Thus the curve (Fig. 2) shows a bulge closely corresponding to the thickness of the calf muscles.

Furthermore, it can be seen from this curve that the peroneal veins are most often thrombosed while the anterior tibial veins show thrombosis only rarely. In all the six limbs in which the anterior tibial veins were occluded, the popliteal vein was also thrombosed. In a considerable number of limbs the proximal end of a

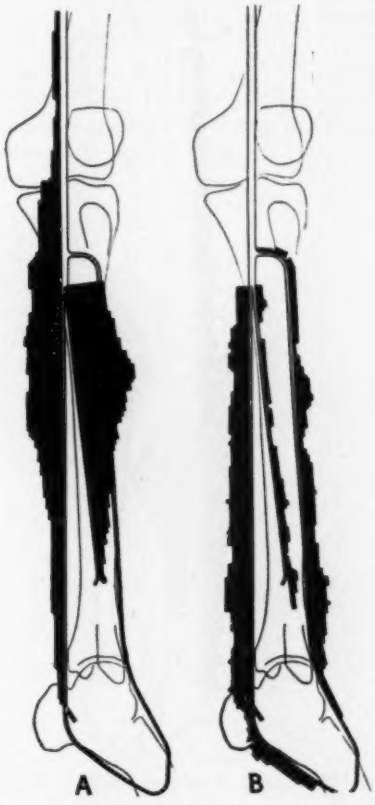


Fig. 1.—Distribution of venous (*A*) and arterial (*B*) thromboses in 22 limbs with coexisting thrombi of both kinds.

thrombus was at the level of the tibial plateau (Fig. 2, arrow). The distal part of the popliteal vein is often double, the two branches joining at this site. Thus the passage from the lower leg into the thigh through at least one of these veins stays open as long as there is a stream from the anterior tibial veins to prevent the thrombi from the posterior veins from growing proximally into the popliteal vein.

From Figure 1 and the table it is seen that arterial and venous thrombosis have a partly opposite localization. The peroneal artery is the most rarely throm-

bosed artery in the leg, while the peroneal veins are the most commonly thrombosed deep veins in the leg. In the posterior tibial vessels, venous thrombosis is mainly to be found in the proximal part, while arterial thrombosis is more evenly distributed and in the larger material could be shown to be more common in the distal half.

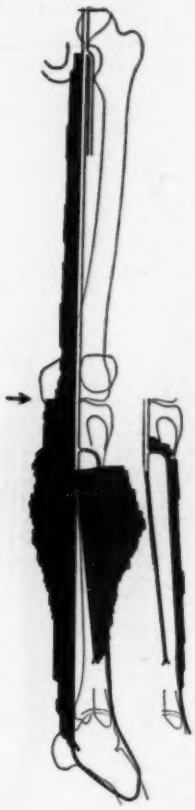

Fig. 2.—Distribution of venous thromboses in 36 limbs.

*The Localization of Arterial and Venous Thromboses in the Lower Leg*

| Thrombosis | | Number of Limbs: Posterior Tibial | Anterior Tibial | Peroneal | Total Number of Limbs |
|---|---|---|---|---|---|
| Arterial | Greatest length of occlusion..... | 57 | 31 | 6 | 94 |
| | Only in ..... | 42 | 17 | 3 | |
| Venous | Greatest length of occlusion..... | 15 | 1 | 20 | 36 |
| | Only in ..... | 2 | 0 | 12 | |

In 22 limbs both arterial and venous thrombi were found to coexist (Fig. 1). In 14 of these, totally different parts of the two kinds of vessels were occluded. In the remaining eight limbs the thrombosed sections to some extent coincided. In no case, however, was the same portion of artery and vein thrombosed.

The presence of arterial thrombosis could not be shown to influence the localization of venous thrombosis, which had the same distribution in the cases with and without arterial thrombosis. Separate curves were drawn and compared.

## SUMMARY

A curve illustrating the way in which venous thrombosis is localized in the deep vessels of the leg has a bulge corresponding to the thickness of the calf muscles, indicating that these muscles are the source of venous thrombi. Arterial and venous thromboses have different and partly opposite localizations in the lower leg, at least in old patients.

# LESIONS OF THE VASA VASORUM AND DISSECTING ANEURYSMS OF THE AORTA

## Analysis of Incidence, Etiological Aspects, Pathogenesis, and Pathological Changes

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IN THE last two decades the subject of dissecting aneurysm of the aorta has aroused considerable interest among clinicians and pathologists, as evidenced by the numerous clinical and investigative publications. Detailed reports of the historical, clinical, and pathological aspects of this condition have been excellently presented by Moritz,[1] Shennan,[2] Sailer,[3] Baer and Goldburgh,[4] David, McPeak, Vivas-Salas, and White,[5] and, more recently, by Bauersfield,[6] and Levinson.[7] Although the pathogenesis is still a matter of conjecture, there is increasing experimental evidence to indicate that the basic pathological change is probably one interfering with the vascular supply of the aortic wall, particularly that of the medial coat (Schlichter [8]).

The present report was stimulated by the contentions of Amromin, Schlichter, and Solway [9] that alteration of the blood supply of the aortic wall induces medial cystic degeneration and rupture of the vasa vasorum resulting in hemorrhagic extravasation and dissection of the media. A careful search of the literature revealed few and diverse observations regarding the pathological changes of the vasa vasorum in cases of dissecting aneurysm of the aorta. Tyson [10] noted intimal proliferation in the vasa vasorum and postulated that obliteration of the vasa produced minute infarcts which weakened the aortic wall. Amromin and associates,[9] in a study of 12 cases of medionecrosis of the aorta, recorded alterations of the

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1. Moritz, A. R.: Am. J. Path. **8**:717, 1932.
2. Shennan, T.: Dissecting Aneurysm, Medical Research Council, Special Report Series No. 193, London, His Majesty's Stationery Office, 1934.
3. Sailer, S.: Dissecting Aneurysm of the Aorta, Arch. Path. **33**:704 (May) 1942.
4. Baer, S., and Goldburgh, H. L.: Am. Heart J. **35**:198, 1948.
5. David, P.; McPeak, E.; Vivas-Salas, E., and White, P. D.: Ann. Int. Med. **27**:405, 1947.
6. Bauersfield, S. R.: Ann. Int. Med. **26**:873, 1947.
7. Levinson, D. C.; Edmeades, D. T., and Griffith, G. C.: Circulation **1**:360,1950.
8. Schlichter, J. G.: Experimental Medionecrosis of the Aorta, Arch. Path. **42**:182 (Aug.) 1946.
9. Amromin, G. D.; Schlichter, J. G., and Solway, A. J. L.: Medionecrosis of the Aorta, Arch. Path. (Oct.) **46**:380, 1948.
10. Tyson, N. D.: Am. J. Path. **7**:581, 1931.

vasa vasorum in seven aortas, consisting of severe narrowing of the lumens of the small and larger vessels of the adventitia due either to marked hypertrophy of the media or to profound splitting and reduplication of the internal elastic membrane. Their study is the first to give particular attention to changes in these nutrient vessels.

The present report is an attempt to confirm the latter changes and elaborate on the lesions of the vasa vasorum at different levels of the aorta and in the involved branches of the aortic trunk. Seven cases of dissecting aneurysm in which autopsy was performed at the Jefferson Medical College Hospital during the period 1947 to 1949 have been selected for this purpose. Before recording our findings it appears pertinent to present a brief analysis of the incidence, the etiologic possibilities, and the pathogenesis of the disease in general.

## INCIDENCE

The true incidence of dissecting aneurysm is not known. A perusal of the literature, however, reveals considerable variation in the frequency at autopsies reported by different authors.[11] The figures ranged from one case in 89 autopsies (1.1%) to one case in 2,500 autopsies (less than 0.1%). The incidence computed from these 14 authors averaged one case in 510 autopsies, or 0.36%.

During the years 1900 to 1949, 24 cases of dissecting aneurysm of the aorta were seen in 6,600 autopsies at the Jefferson Medical College Hospital. This resolves into an incidence of one case in 275 autopsies, or 0.4%. It is interesting to note here that 18 of the 24 cases were seen during the years 1936 to 1949. A similar observation is recorded by David and associates,[5] who found 17 of their 30 cases after 1936. This is due in no small measure to Shennan's classic publication in 1934, which brought this entity to the foreground.

The sex incidence shows a preponderance in males. Some of the recorded male-to-female ratios are as follows: Glendy, Castleman, and White,[12] 15:4; Flaxman,[11c] 13:6; Ritvo and Votta, [11i] 3:1; Mote and Carr,[11f] 52:8; Baer and Goldburgh,[4] 29:15, and Bauersfield,[6] 11:4. The average incidence compiled from different authors is 3:1 in favor of males. In our series of 24 patients 19 were men and five women.

The ages of the patients in our series ranged between 26 and 72 yr. with an average of 52 yr. Twenty-two patients were 40 yr. of age or more, and 13 of these were between the ages of 41 and 60 yr. This is in accordance with most authors, who have reported the greatest incidence to be between the ages of 40 and 60. In the series reported by Glendy and associates [12] 18 of the 19 patients

11. (*a*) Crane, P. M., in Medical Papers Dedicated to Henry Asbury Christian, Physician and Teacher, in Honor of His 60th Birthday, Feb. 17, 1936: From His Present and Past Associates and House Officers at the Peter Bent Brigham Hospital, Boston, Mass., Baltimore, Waverly Press, Inc., 1936, p. 142. (*b*) Farinacci, C. A.: Bull. School Med. Univ. Maryland **19**:47, 1934. (*c*) Flaxman, N.: Am. Heart J. **24**:654, 1942. (*d*) Gouley, B. A., and Anderson, E.: Ann. Int. Med. **14**:978, 1940. (*e*) Logue, R. B.: Am. J. M. Sc. **206**:54, 1943. (*f*) Mote, C. D., and Carr, J. L.: Am. Heart J. **24**:69, 1942. (*g*) Palmer, J. D., and Mathison, A. K.: Canad. M. A. J. **55**:585, 1946. (*h*) Peery, T. M.: Am. Heart J. **12**:650, 1936. (*i*) Ritvo, M., and Votta, P. J.: Am. J. Roentgenol. **52**:583, 1944. (*j*) Walker, C., and Walker, L.: Brit. M. J. **2**:200, 1919 (*k*) Weiss, S.: M. Clin. North America **18**:1117, 1935. (*l*) Shennan.[2] (*m*) Sailer.[3] (*n*) David and others.[5]

12. Glendy, R. E.; Castleman, B., and White, P. D.: Am. Heart J. **13**:129, 1937.

were over 40 yr. of age; Baer and Goldburgh [4] recorded the ages of 42 of 44 patients as over 40, and Schnitker and Bayer,[13] in their large series of 580 cases, noted that 439 patients were over 40.

### ETIOLOGICAL ASPECTS

No determinant etiological factor or disease process has been demonstrated as consistently producing the pathological changes of dissecting aneurysm. A myriad of causes believed responsible for its production is reported in the literature. For the sake of convenience, these will be discussed under the following five headings: congenital, inflammatory, degenerative and metabolic, mechanical, and experimental.

*Congenital Anomalies.*—Coarctation of the aorta and arachnodactyly are two congenital anomalies that have been reported to be associated with dissecting aneurysms in persons dying of the latter condition. In a review of 200 cases of coarctation of the aorta, Hamilton and Abbott [14] found that 33, or 16.5%, of the patients had died from dissecting aneurysm. Schnitker and Bayer,[13] in their studies of dissecting aneurysm in 141 persons under the age of 40 yr., reported that 45, or 31.9%, had either true coarctation or definite narrowing of the aorta. Two persons who presented arachnodactyly and who died suddenly of dissecting aneurysm were described by Tobin, Bay, and Humphreys,[15] and another who had arachnodactyly and a dislocated lens and who died of rupture of a dissecting aneurysm of the aorta was reported by Etter and Glover.[16] Baer, Taussig, and Oppenheimer [17] found cystic medionecrosis in two cases of congenital aneurysmal dilatation of the aorta associated with arachnodactyly.

From these observations there is evidence to suggest that a developmental defect of the aorta may be a predisposing factor in dissecting aneurysm. It is conceivable that in coarctation of the aorta the congenital narrowing of the aorta may be associated with a defect in the vascularity of the aortic wall which may produce cystic necrosis of the media with hemorrhagic extravasation and dissection. The occurrence of dissecting aneurysm in cases of arachnodactyly is more difficult to explain. Since congenital anomalies, when they occur, are usually multiple, it is perhaps not too presumptuous to postulate that an associated anomaly of the wall of the aorta may be the initiating factor. This finds support in Tobin's [15] report of four cases of arachnodactyly in which other anomalous defects of the heart were present.

*Inflammatory Lesions.*—Syphilis is the most frequently incriminated inflammatory lesion reported as the probable cause of dissecting aneurysm. Recent investigations, however, do not show any considerable difference in the incidence of dissecting aneurysm among syphilitic and nonsyphilitic patients. Among 141

13. Schnitker, M. A., and Bayer, C. A.: Ann. Int. Med. **20**:486, 1944.

14. Abbott, M. E., and Hamilton, W. F.: Am. Heart J. **3**:381, 1928.

15. Tobin, J. R., Jr.; Bay, E. B., and Humphreys, E. M.: Marfan's Syndrome in Adult; Dissecting Aneurysm of the Aorta Associated with Arachnodactyly, Arch. Int. Med. **80**:475 (Oct.) 1947.

16. Etter, L. E., and Glover, L. P.: Arachnodactyly Complicated by Dislocated Lens and Death from Rupture of Dissecting Aneurysm of the Aorta, J. A. M. A. **123**:88 (Sept. 11) 1943.

17. Baer, R. W.; Taussig, H. B., and Oppenheimer, E. H.: Bull. Johns Hopkins Hosp. **72**:309, 1943.

cases of dissecting aneurysm. Schnitker and Bayer [18] found only 5 cases, or 3.5%, with a history of syphilis. Shennan,[2] with a series of 218 cases, reported syphilis in 10%. McGeachy and Paullin [18] discovered syphilis in six of 127 cases (4.7%).

Sailer [8] and Klotz and Simpson [19] maintained that the very nature of the syphilitic process with the development of fibrous connective tissue in the aorta hindered, rather than favored, the development of dissection. These authors questioned seriously the possibility of syphilis being an active cause of dissecting aneurysm. Certainly, in the larger series reported by Schnitker and Bayer,[18] Shennan,[2] and McGeachy and Paullin [18] the incidence of syphilis was very low among patients in whom dissecting aneurysm developed.

Various infectious diseases have been incriminated as causes of dissecting aneurysm but there is not sufficient substantiation of these charges. Rheumatic fever was reported as the cause of dissecting aneurysm in a single case recorded by Gray and Aitkin.[20]

It is doubtful, from the evidence at hand, that inflammatory lesions, such as those of syphilis or infectious diseases, play an intimate causal role in dissecting aneurysm. That infection may predispose an individual to the disease cannot be denied, but the fact that dissecting aneurysm occurs mostly in patients more than 40 yr. of age without a history of infection mitigates against its being the sole factor.

*Degenerative and Metabolic Causes.*—Arteriosclerosis is the most common etiological factor cited in dissecting aneurysm. In the light of our present understanding of the pathology of the latter disease, it is questionable whether arteriosclerosis as such can be considered a cause. To explain the pathogenesis of dissecting aneurysm on the basis of arteriosclerosis it must first be assumed that the site of greatest weakness in the aortic wall is the media, and secondly that dissection starts with an intimal tear followed by hemorrhagic penetration from the aortic lumen into the media. It is generally known that arteriosclerosis primarily involves the subintimal connective tissue and extends to involve the media adjacent to the intima. The latter change does not occur in dissecting aneurysm, since the outer third of the media is usually the site of more extensive cystic medionecrosis. Moreover, there are numerous reports of dissecting aneurysm with complete absence of an intimal tear and communication between the intramural hematoma and the aortic lumen. Tyson [10] reported three of four cases having no intimal tear, while Mote and Carr [11f] found four in their series of 60 cases. Three other cases were reported by Reisinger,[21] and Hamburger and Ferris.[22] Finally, Bauersfield [6] indicated that arteriosclerosis is an unlikely etiological factor because it is least extensive in the

18. McGeachy, T. E., and Paullin, J. E.: Dissecting Aneurysm of the Aorta, J. A. M. A. **108**:1690 (May 15) 1937.

19. Klotz, O., and Simpson, W.: Am. J. M. Sc. **184**:455, 1932.

20. Gray, S. H., and Aitkin, L.: Late Gross Lesions in Aorta and Pulmonary Artery Following Rheumatic Fever, Arch. Path. **8**:451 (Sept.) 1929.

21. Reisinger, J. A.: Dissecting Aneurysm of the Aorta, Arch. Int. Med. **65**:1097 (June) 1940.

22. Hamburger, M., and Ferris, E. B.: Am. Heart J. **16**:1, 1938.

ascending aorta, where most of the dissecting aneurysms occur, and because intimal tears are seen between atherosclerotic plaques rather than in them.

Since Erdheim [23] described the cystic degenerative changes in the medial coat of the aorta, most observers have regarded idiopathic cystic medionecrosis as the pathologic change in dissecting aneurysm predisposing to eventual hemorrhage and dissection.

It is interesting to note that in women patients under 40 yr. of age in whom dissecting aneurysm develops, there appears to be some relationship with pregnancy. Schnitker and Bayer,[13] in their series of 141 cases of dissecting aneurysm in persons under the age of 40 yr., found that 49 of the patients were women, 24 of whom were pregnant. The stage of the pregnancy was during labor in 2 cases, post partum in 2, and ante partum in 20. McGeachy and Paullin [18] discovered six pregnant women among 26 patients with dissecting aneurysm. An explanation of this relationship of pregnancy and dissecting aneurysm is wanting.

*Mechanical Factors.*—Because of the observation that most of the patients dying from dissecting aneurysm had elevated blood pressure, it was believed for a long time that hypertension played a primary role in the causation of the disorder. Klotz and Simpson,[19] in 1932, subjected aortas to various pressures and found that a pressure of 1,000 mm. of mercury was required before rupture of the aorta occurred. Robertson and Viner-Smith [24] inserted a needle into the media of the aorta at autopsies and measured the applied pressure necessary for dissection. They concluded that the pressure required to produce medial dissection was far in excess of aortic blood pressure even in the most severe forms of hypertension in the human. They were further convinced of the virtual impossibility of producing a dissecting aneurysm by excess of blood pressure during life, through an intimal tear, into normal media. From these observations it is apparent that hypertension of itself does not cause dissecting aneurysm and at most probably plays a secondary role. This secondary role is believed by some observers to be the facilitation of the dissection after an intramural hematoma forms in the aorta.

The relationship of trauma and exertion to dissecting aneurysm is still disputed. Weiss [11k] was of the opinion that most cases reported following trauma were not true cases of dissecting aneurysm. In the matter of exertion, there is no substantial evidence to indicate that it has any etiological significance. Although it is true that in many patients the signs and symptoms of dissecting aneurysm develop following exertion, it is equally true that many are asleep or at rest when the symptoms develop.

*Experimental Evidences.*—Various experimental procedures have been devised to produce the changes of idiopathic cystic medionecrosis in the aorta. Among these have been the use of diphtheria toxin (Duff [25]), nicotine, large doses of cholesterol, epinephrine, sublethal doses of histamine, tyramine, and other agents (Sailer [3]). Many of these experiments produced inconsistent results, so that there is insufficient

23. Erdheim, J.: Arch. path. Anat. **276**:187, 1930.

24. Robertson, J. S., and Viner-Smith, K.: J. Path. & Bact. **60**:43, 1948.

25. Duff, G. L.: Medial Degeneration in Aorta of Rabbit Produced by Diphtheria Toxin, Arch. Path. **13**:543 (April) 1932.

evidence to prove that any of the above-mentioned agents plays a definite etiological role. However, it is generally agreed that the defect in the aortic wall is cystic degeneration of the media, and therefore it is not unlikely that the use of the agents mentioned may have interfered with the oxygenation and nutrition of the aortic wall, causing cystic medionecrosis. The most convincing experimental study, in this respect, is that carried out by Schlichter,[6] who seared the adventitia of the aorta in dogs and after a period of one to six weeks studied the histopathological changes in the aorta. He found definite cystic degenerative changes in the media and reported one animal dying from rupture of the aorta. Necrotic and degenerative changes of the media of the abdominal aorta and peripheral arteries also have been produced in rabbits by crushing the vascular wall, by dissecting the adventitia, by applying acids and thermocautery to the outside of the vascular wall, and by sheathing the vascular wall in paraffin (Schlichter.[6]).

## PATHOGENESIS

On the basis of available experimental, clinical, and histopathological data, the pathogenesis of dissecting aneurysm may be accounted for in theory as follows: A weakened condition of the medial coat (caused by factors as yet undetermined) is associated with cystic medionecrosis, rupture of the vasa vasorum, and resultant intramural hematoma. The internal pressure of the aorta is exerted on the hematoma, which then spreads along the line of least resistance. As the hemorrhagic extravasation from the ruptured vasa vasorum continues, further dissection occurs, and ultimately the hemorrhage destroys the intima and communicates with the aortic lumen. The tear in the intima facilitates flowing of aortic blood into the dissected media and this, aided by the intra-aortic pressure, may produce further and extensive dissection of the medial coat.

## PATHOLOGICAL CHANGES

The general pathological changes of dissecting aneurysm have been excellently presented by Sailer,[3] Moritz,[1] and others, and these will not be discussed here. The cystic degenerative changes of the media, however, suggest a possible relationship with alterations in the vasa vasorum, and accordingly, this report is directed at a study of the changes in the vasa at different levels of the aorta in seven cases of dissecting aneurysm.

*Methods.*—In seven cases of dissecting aortic aneurysm the entire aorta was examined grossly for (1) presence, location, extent, and nature of the intimal tear, (2) direction and extent of the medial dissection, and (3) presence and location of the adventitial perforation. Representative sections for histological study were obtained from (1) the root, arch, thoracic portion, and abdominal portion of the aorta not showing macroscopic dissection, (2) the area showing the dissection, and (3) any involved arterial branches. The specimens, which were previously fixed in Klotz solution, were further fixed in 10% formaldehyde solution, sectioned, and stained with hematoxylin and eosin, Bodian's silver stain, Weigert's elastic stain, Masson's trichrome stain, polychrome methylene blue, Van Gieson's stain, and safranine O. Seven aortas taken from patients selected at random and dead of other causes were submitted to a similar study.

*Observations.*—Grossly, all seven cases of dissecting aneurysm of the aorta demonstrated the characteristic appearance of the disorder, consisting of prominent splitting of the media and intramural hemorrhage between the split portions of the

medial coat. They differed only in the site of the intimal tear, the site of the adventitial perforation, the extent of the medial dissection, the degree of involvement of the circumference of the aorta, and the involvement of the arterial branches of the aortic trunk. In four cases the adventitial tear resulted in massive hemopericardium; in one case it produced an extensive hemorrhage involving the pleural cavity, while the remaining two cases eventuated in serious retroperitoneal hemorrhage. In six cases the dissection extended from near the root of the aorta to the iliac bifurcation (Fig. 1). In two of these six cases the aneurysm extended into the right external iliac artery for several centimeters, and in one case, into the left external iliac artery. In the remaining case, medial splitting involved only 6 cm. of the aorta, but perforation of the adventitia occurred in the pericardial cavity, resulting in massive hemopericardium.

Fig. 1.—A dissecting aneurysm showing an intimal tear in the ascending aorta and an intramural hemorrhage involving the ascending, thoracic and abdominal portions of the aorta.

Of the seven cases, three demonstrated involvement of the arterial branches. In the first instance, both the innominate and the left common carotid artery showed medial dissection and hemorrhage for a short distance from their origin. A second case disclosed involvement of the innominate artery for a distance of 4 cm. (Fig. 2) and of the left renal artery for 3 cm. In this particular case, acute diffuse cortical necrosis of the left kidney eventuated from occlusion of the renal artery. A third case demonstrated involvement of the superior mesenteric artery for a distance of 1.8 cm. beyond its origin.

Histologically, sections from the involved portion of the aorta showed medial necrosis with massive intramural hemorrhage in all seven cases in this series. In the areas of intact media, alterations fulfilling the established criteria for idiopathic cystic medionecrosis were observed (Fig. 3). These alterations consisted of homogeneous, pale-staining, basophilic acellular areas between the muscle and elastic fibers in sections stained with hematoxylin and eosin, and of rose-colored areas in sections stained with polychrome methylene blue.

At different levels of the aorta, in both the involved and the uninvolved portions, the vasa vasorum revealed alterations of varying types and intensity in all seven

Fig. 2.—A dissecting aneurysm showing an intimal tear in the ascending aorta and dissection of the medial coat of the innominate artery producing occlusion of the lumen.

cases. In general, the changes observed in the vasa vasorum were prominent in the portions of the aorta showing hemorrhage and dissection or medial necrosis.

At the site of the intimal and adventitial tears, where the pathologic changes in the media were most severe, extensive hemorrhage and necrosis obscured careful observation of the vasa vasorum. Where the vasa vasorum could be visualized, they showed obvious engorgement. In two cases, in which dissection was evidently of a slowly progressive nature, pronounced capillary and fibroblastic proliferation associated with diffuse infiltration of lymphocytes, plasma cells, and monocytes were observed. In two other cases, conspicuous hypertrophy of the media of the small arteries at the site of the intimal tear was noted (Fig. 4).

In sections of the aorta taken at other levels the small arteries of the vasa vasorum, for the greater part, showed the least extensive changes. Alterations consisting of medial hypertrophy and splitting of the internal elastic membrane were

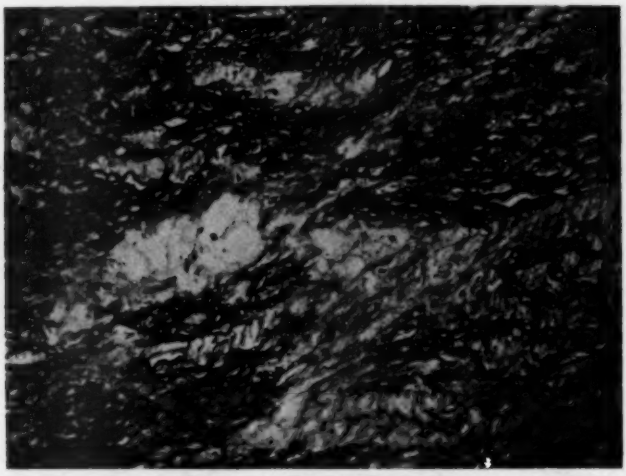

Fig. 3.—Aorta showing idiopathic cystic medial necrosis. Weigert's stain; × 100.

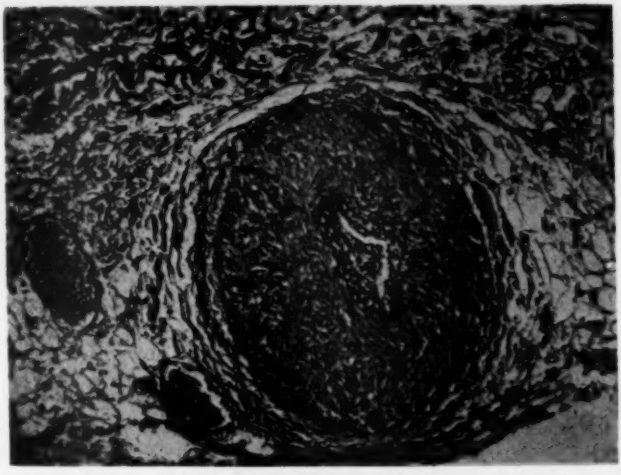

Fig. 4.—A small artery of the adventitia in a case of dissecting aneurysm. It shows conspicuous medial hypertrophy. Weigert's stain; × 100.

observed. Conspicuous and diffuse changes, however, were noted in the arterioles. The walls of many of these vessels were thickened because of medial hypertrophy, intimal proliferation, or swelling of the endothelial cells. In one case several arterioles exhibited narrowing of their lumens to such a degree as to form transverse or stellate

slits. In a few arterioles hyalinization of the media was also noted. These arteriolar changes were observed in all seven cases and were present to a greater or lesser extent in all sections taken at the different levels (Figs. 5 to 7*A*).

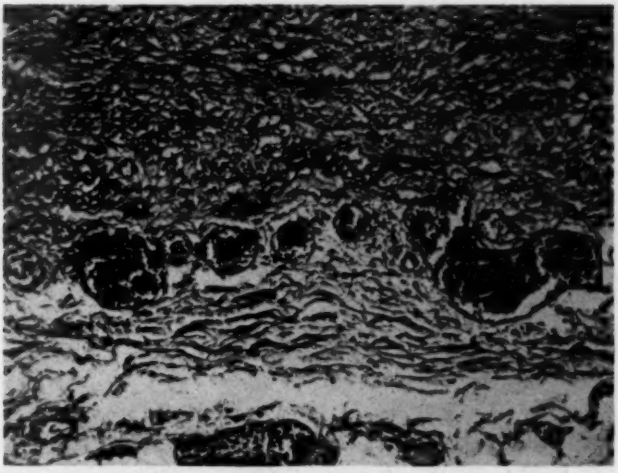

Fig. 5.—Normal aorta showing vasa vasorum. Hematoxylin and eosin; × 100.

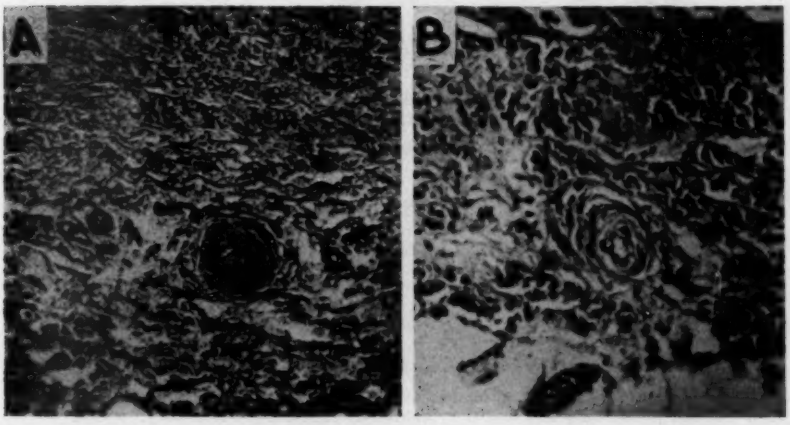


Fig. 6.—*A*, dissecting aneurysm showing adventitial fibrosis, medial hypertrophy of a small artery and endothelial proliferation of arterioles. Hematoxylin and eosin; × 100.
*B*, arteriole showing medial hypertrophy. Hematoxylin and eosin; × 200.

Capillary changes were also noted in all the seven cases. The alterations consisted chiefly of endothelial swelling or proliferation producing partial to complete occlusion of the capillary lumen. In many of the capillaries, particularly those in the

vicinity of the medial dissection, the lumens were narrowed by four to five endothelial cells lining the capillary channels. In others, simple swelling of the endothelial cells caused obliteration of the lumen (Fig. 7*B*).

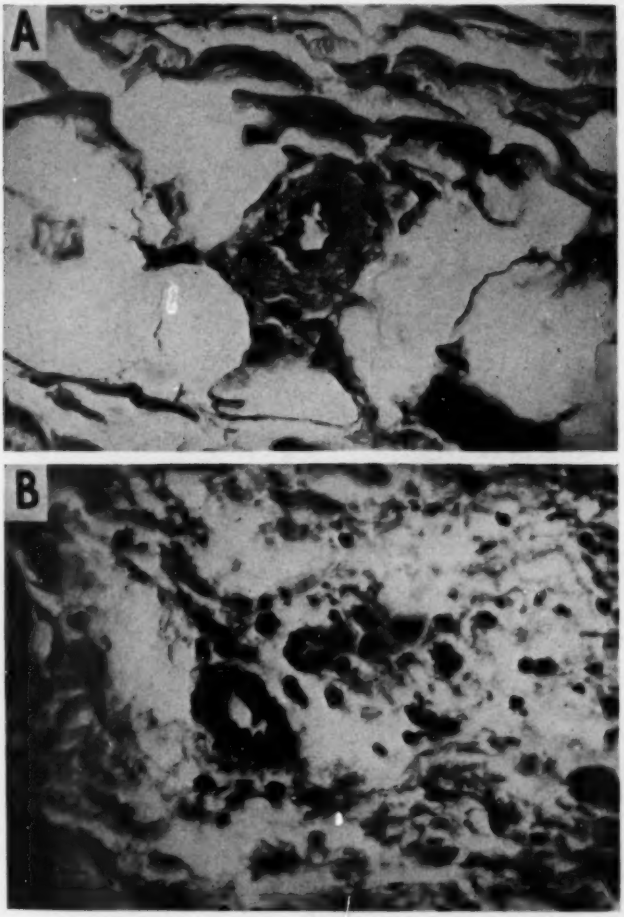


Fig. 7.—*A*, arteriole in the adventitia of an aorta undergoing dissecting aneurysm. It shows intimal proliferation and medial hypertrophy. Hematoxylin and eosin; × 400.

*B*, marked intimal proliferation of a capillary and of an arteriole in the adventitia in the region of a dissecting aneurysm. Hematoxylin and eosin; × 200.

Studies of sections of the involved branches of the aorta included the innominate and superior mesenteric arteries. These had the same changes in the vasa vasorum as seen in the aorta. A few of the small arteries disclosed medial hypertrophy and

splitting of the internal elastic membrane. The arterioles showed hyalinization of the media, intimal proliferation, and swelling of the endothelial cells. Capillary alterations consisted of endothelial swelling with partial obliteration of the lumen.

The changes noted in the aorta and involved arterial branches in cases of dissecting aneurysm were not noted in seven control aortas taken at random from patients dead of other diseases. Hyalinization of the media was noted in small arteries of the vasa vasorum in two of the controls, but was not conspicuous. The arteriolar and capillary alterations were absent.

## COMMENT

The results of this study conform with the observations made by Amromin, Schlichter, and Solway [9] and Tyson [10] that changes are present in the vasa vasorum in dissecting aneurysm of the aorta. Although Tyson noted only intimal proliferation in the vasa vasorum, the alterations are not limited to this change. In accordance with Amromin and associates, the changes we observed consisted of medial hypertrophy and splitting of the internal elastic membrane in the small arteries, medial hypertrophy with intimal proliferation and endothelial swelling in the arterioles, and endothelial proliferation and swelling in the capillaries. Of these vascular changes we believe those involving the arterioles are of primary significance and those involving small arteries and capillaries are of secondary importance. The end result of these changes is a narrowing or occlusion of the lumen in the vessels comprising the vasa vasorum.

Tyson,[10] on noting the intimal proliferation of the vasa vasorum in cases of dissecting aneurysm, believed that arteriosclerosis, thrombosis, and low-grade inflammation could obliterate the vasa to produce minute areas of infarct and weaken the aortic wall. Amromin, on the other hand, was of the opinion that the alterations in the vasa vasorum produce ischemia followed by cystic degenerative changes of the media.

At the present time there are insufficient data on the alterations of the vasa vasorum for us to attempt to correlate the pathological changes with etiological factors. The findings outlined, however, suggest that a host of etiological factors may produce obliteration of the vasa vasorum with ensuing ischemia and idiopathic cystic medionecrosis. From the experimental point of view the work of Schlichter [8] and others tends to support this contention. As already stated, these workers altered the vascular supply of the aorta by various methods in animals and produced changes consistent with idiopathic cystic medionecrosis of the aorta.

The question of why alterations of the vasa vasorum occur cannot be answered at the present time. Neither can the nature of cystic degeneration of the media be explained. Endocrine factors relating to degeneration of connective tissue are too little understood to be of any help. Metabolic factors, probably occurring in pregnant women and in patients in the older age group dying of this disorder, are likewise not well understood. Thus, the etiological aspects of this disorder must await elucidation at some future date. It is hoped, however, that this report will stimulate further studies of larger series of dissecting aneurysms in order that the changes in the vasa vasorum may be further evaluated and correlated with the pathogenesis of this disorder.

SUMMARY

The true incidence of dissecting aneurysm is not known, but the autopsy incidence averages one case in 500, or 0.36%. The disease is three times more prevalent in men than in women and occurs most commonly between the ages of 40 and 60.

The cause is not known. It is believed, however, that a weakened condition of the medial coat associated with cystic medionecrosis is followed by rupture of the vasa vasorum with intramural hemorrhage which spreads in the media to involve variable lengths of the aorta and its branches.

Grossly, the aorta is characterized by prominent splitting of the media with intramural hemorrhage between the split portions of the aortic wall. Intimal tear and adventitial perforation are frequently present. Involvement of the different arterial branches of the aorta may be observed. Histopathological changes consisting of medial necrosis with massive intramural hemorrhages are the most prominent features of dissecting aneurysm. Alterations of the intact media consist of cystic medionecrosis. In this report, particular reference is made to the alterations of the vasa vasorum in seven cases of dissecting aneurysm in which autopsies were made at the Jefferson Hospital. These changes consist of (1) medial hypertrophy and splitting of the internal elastic membrane of small arteries, (2) medial hypertrophy with intimal proliferation and endothelial swelling of the arterioles, and (3) endothelial swelling and proliferation of the capillaries. Of these, the arteriolar changes are by far the most prominent. Similar changes were not found in seven aortas taken at random from persons dead of other causes.

# MALIGNANT HYPERTENSION FOLLOWING THE ADMINISTRATION OF CORTISONE IN PERIARTERITIS NODOSA

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THE TREATMENT of periarteritis nodosa with cortisone or with adrenocorticotropic hormone (ACTH) has a sound experimental background. Selye [1] has shown that lesions similar to periarteritis nodosa may be produced consistently in animals with mineralocorticoids (desoxycorticosterone) and that production of these lesions may be inhibited by glucocorticoids (cortisone). Rich and Gregory [2] have produced periarteritic changes in rabbits by means of horse-serum sensitization. In other experiments Rich, Berthrong and Bennett [3] have shown that cortisone or ACTH prevents development of these lesions in a significant number of animals. Clinically, there have been several reports on the beneficial effects of cortisone (or ACTH) in cases of periarteritis nodosa.[4] These effects are prompt and dramatic but apparently temporary; eventual clinical recurrence sometimes occurs. In a group of five patients with periarteritis nodosa treated with cortisone and ACTH [4a] two died in cardiorenal failure despite initial improvement. At autopsy there was complete healing of all arterial lesions, but this process had resulted in obliteration of these vessels and widespread visceral infarction. In a third patient, severe and progressive hypertension developed following cortisone therapy, with, however, subsequent improvement of the hypertension. Malig-

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1. Selye, H.: The Physiology and Pathology of Exposure to Stress, Montreal, Acta Endocrinologica, Publisher, 1950.

2. Rich, A. R., and Gregory, J. E.: Experimental Demonstration That Periarteritis Nodosa Is a Manifestation of Hypersensitivity, Bull. Johns Hopkins Hosp. **72**:65-88, 1943.

3. Rich, A. R.; Berthrong, M., and Bennett, J. L.: The Effects of Cortisone upon the Experimental Cardiovascular and Renal Lesions Produced by Anaphylactic Hypersensitivity, Bull. Johns Hopkins Hosp. **87**:549-568, 1950.

4. (*a*) Shick, R. M.; Baggenstoss, A. H., and Polley, H. F.: The Effects of Cortisone and ACTH on Periarteritis Nodosa and Cranial Arteritis: Preliminary Report, Proc. Staff Meet., Mayo Clin. **25**:135, 1950. (*b*) Goldman, R.; Adams, W. S.; Beck, W. S.; Levin, M.; Bassett, S. H., and White, A.: The Effects of ACTH on One Case of Periarteritis Nodosa, Proc. 1st Clin. ACTH Conf., Chicago, Philadelphia, The Blakiston Company, 1950, pp. 437-445. (*c*) Stillman, J. S., in discussion on Goldman and others,[4b] pp. 445-446.

nant hypertension occurring in the course of cortisone treatment has been reported occasionally. A recent report [5] of malignant hypertension in a patient treated with cortisone for scleroderma was confirmed by autopsy. Having in mind the increasing use of cortisone and the need to evaluate its morphologic effects, we offer the following detailed autopsy report.

## CLINICAL HISTORY

A 28-year-old white machine operator was admitted to the Veterans Administration Hospital, Bronx, N. Y., in September, 1950, complaining of fever and soreness of his calf muscles. The illness was of four months' duration and had begun with a severe sore throat. This was followed within three weeks by a rash (which he described as hives), swelling of the hands, and migrating polyarthritis of toes and ankles. He was admitted to another hospital, where the following observations were recorded: 1. The physical examination showed only tenderness of joints and bilateral tenderness of the costovertebral angles. 2. Beta hemolytic streptococcus was isolated from the throat. 3. The erythrocyte sedimentation rate was sharply elevated: 125 mm. per hour. 4. Trichinella skin tests were negative, but a precipitin test was positive (1:1,280). 5. The blood pressure was 120/70. 6. Examinations of the urine and the bone marrow, blood cultures, and muscle biopsy gave negative results. The patient was treated with salicylates, penicillin, aureomycin, and chloramphenicol (chloromycetin®), without effect, and was discharged unimproved, his disease undiagnosed.

When first seen at the Veterans Administration Hospital the patient had a temperature of 101 F., a pulse rate of 80, and a blood pressure of 140/90. The only abnormal finding on physical examination was tenderness of the calf muscles and joints.

The white blood cell count was 11,000 (neutrophils 72%, lymphocytes 15%, monocytes 9%, eosinophils 2%, basophils 2%); the erythrocyte count was 3,700,000; the hemoglobin content, 12 gm. The erythrocyte sedimentation rate, corrected, was 26 mm./hr. (Wintrobe). The urine had a specific gravity of 1.012, no albumin, no sugar, and microscopic examination gave negative results. The antistreptolysin titer was 300-400. Agglutination tests for Brucella, Salmonella typhosa, and Salmonella paratyphi were negative. The Kahn test was negative. Total serum proteins amounted to 8.0 gm., with albumin 4.3 and globulin 3.7 gm., per 100 cc. An electrocardiogram was normal.

A working diagnosis of acute rheumatic fever was made, and sodium salicylate therapy was started. Since this was without effect, cortisone therapy was instituted. He received 300 mg. of cortisone acetate the first day, 200 mg. the second day, and 100 mg. the third day—a total of 600 mg.

At 7:10 p. m. on the day of the last cortisone injection the patient had a generalized convulsion which lasted for approximately 30 sec. Within half an hour he was well oriented and coherent. The right pupil was larger than the left, and upper-motor-neuron signs were elicited. These signs changed rapidly, became equivocal, and disappeared within a few hours. The blood pressure was 178/110, the pulse rate 128, and the temperature 103 F. Anuria supervened for the next 15 hr., after which there was profuse diuresis. Cortisone therapy was discontinued, and the patient was treated symptomatically. The following day he had two more generalized convulsions. The spinal fluid pressure at this time was 280 mm. of water. Examination of the fluid showed no abnormalities. Five days after the cessation of cortisone therapy, a fourth convulsive episode occurred. The last three convulsions were exactly like the original one, being characterized by tonic and clonic movements and followed by rapidly changing neurologic signs, which completely disappeared in a few hours. He became lucid and cooperative after the first three seizures, but after the last convulsion he was thenceforth semistuporous and disoriented except for momentary improvement.

The first urine following the period of anuria showed albumin (4+), granular casts and 6 to 8 leukocytes per high-power field. On this day the serum potassium was 3.82 mEq./l., and during the remainder of the patient's course the determinations varied from 2.40 to 3.95 mEq./l.

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5. Sharnoff, J. G.; Carideo, H. L., and Stein, I. D.: Cortisone Treated Scleroderma, J. A. M. A. **145**:1230-1232 (April 21) 1951.

The serum sodium ranged from 130 to 142 mEq./l. during this time. 17-Ketosteroids fluctuated from 9 mg./24 hr. to 19.7 mg./24 hr. Repeated fasting blood-glucose determinations remained within normal limits (77 to 125 mg. per 100 cc.). Blood cultures were repeatedly sterile. During this period the urine continued to show albumin (3 to 4+), granular casts, and 8 to 12 white blood cells and 25 to 30 erythrocytes per high-power field. The specific gravity of the urine varied from 1.008 to 1.018. The blood urea nitrogen rose to a peak of 48 mg. per 100 cc. shortly after the convulsions and then fell by lysis to normal in about 10 days and did not rise again (the last blood-urea-nitrogen determination was done five days before death). Serum chlorides varied from 73 to 104 mEq./l., and at the same time the $CO_2$-combining power ranged from 64 to 90 volumes per cent. Terminally the erythrocyte count dropped to 2,500,000, with a hemoglobin content of 7.3 gm. The total serum proteins were 6.3 gm., with an albumin-globulin ratio of 3.9 : 2.4.

Toward the end of his hospital course the patient's blood pressure fluctuated from 210/160 to 250/180. A transient maculopapular rash appeared on the back, in the axillae, and on the neck. The fundi were examined frequently and were normal until the fourth day following cortisone therapy, when small scattered hemorrhages and papilledema were noted. Changes in the eyegrounds then became progressively more severe, culminating in complete bilateral retinal detachment within a period of 10 days. Mild ankle and pretibial edema was noted at this time. During the remainder of his life the patient pursued a downhill course, becoming increasingly lethargic, disoriented, and weak. During the last month he complained little of soreness of muscles, and joints, these symptoms being replaced by severe intermittent headache, pain in the eyes, and recurrent generalized abdominal pain. The latter complaint occurred at intervals when he had hypochloremia and was relieved by the administration of saline solution. The urinary findings persisted, and the blood pressure remained elevated. He became so weak that he could no longer maintain an adequate airway. During the last 20 hr. of life there was a urinary output of only 450 cc. in spite of the parenteral administration of adequate amounts of whole blood and saline solution. He died in a state of pulmonary edema on Nov. 16, 1950, 54 days after admission, 44 days after cortisone therapy.

## NECROPSY

*Gross Examination.*—There was a small amount of clear serous fluid in each pleural space. The lungs showed intense congestion, edema, and irregular areas of consolidation in all lobes. The heart weighed 510 gm. and showed moderate hypertrophy of the left ventricle without dilatation of the auricles or the ventricles. The valves were negative, except for the mitral valve, which showed five or six soft white nodules, measuring approximately 0.2 cm. in diameter, on the free edge of each leaflet. The myocardium was negative. The liver weighed 2,700 gm. and showed severe passive congestion. The gastrointestional tract, spleen, pancreas, thyroid, and musculoskeletal system showed no gross abnormalities. Both adrenals were very thin and closely adherent to the upper poles of the kidneys and in many areas could not be readily separated from the kidneys. The area covered by the adrenals was not excessive. The kidneys each weighed 300 gm. and were alike in appearance. The capsules stripped with ease except in areas where the adrenals were densely adherent. The cortical surfaces exhibited large dark-red depressed zones of irregular size and shape which formed geographic patterns contrasting sharply with the intervening pale, tan-colored, swollen surface. The cut section showed corresponding areas of hemorrhage with thinning of the cortex and intervening areas of pale, swollen parenchyma. Pinpoint hemorrhages were scattered throughout the cortex. The renal arteries and veins were normal. The medullae, calices, pelves and ureters, as well as the rest of the genitourinary tract, were not remarkable. The brain weighed 1,500 gm. and contained two zones of organizing hemorrhage. One was located in the subcortical white matter of the right middle frontal gyrus and measured approximately 1 cm. in diameter. The

other hemorrhage was located in the cortex and subcortical white matter of the left temporal, parietal, and occipital lobes and measured 7 by 3.5 cm. The eyes were not examined.

*Microscopic Examination.*—The predominant feature was a widespread vascular lesion involving many viscera, as well as skeletal muscle. The vessels affected were generally small to medium-sized arteries and occasionally arterioles. The lesions were focal, affecting only small segments of vessels or even a portion of the vessel seen in cross section. Acute and healing stages of the lesion were distinguished. In the acute phase there was fibrinoid, eosinophilic necrosis of the vessel wall associated with an inflammatory infiltration and mild intimal proliferation (Fig. 1*D* and *E*). The necrosis did not always extend throughout the entire vessel wall. The infiltrate consisted of neutrophils, lymphocytes, and occasionally eosinophils; it involved the wall of the vessel as well as the perivascular space. In the healing stage (Fig. 1*A, B* and *C*) there was fibrous proliferation of intima and adventitia. Thrombosis with organization and recanalization was frequently observed. A panvascular and a perivascular inflammatory infiltrate was often noted; unlike the infiltrate in the acute phase, it was often discrete and generally consisted of lymphocytes. Disruption and destruction of portions of the internal elastic lamina and aneurysmal dilatations were frequent (Fig. 1*A* and *B*). The lesions described represent the two extremes of the process; a large variety of intermediate stages were noted. They are all characteristic of periarteritis nodosa. In some organs these vascular lesions, although present, did not result in any recognizable parenchymal change, namely, in the testes, the pancreas, the esophagus, the stomach, the adrenal gland (capsule) and striated muscle. In other organs these lesions produced parenchymal changes. In the heart the myocardium was irregularly infiltrated with neutrophils, lymphocytes, and eosinophils. In the liver there were many areas of dense neutrophilic infiltration.

In the kidney, periarteritic lesions, principally in the healing phase, involved the large vessels (arcuate and interlobular arteries). Complete healing with organization and recanalization was often present. In the cortex there were irregular areas of scarring with mild stromal fibrosis and lymphocytic infiltration. Many tubules were dilated and contained eosinophilic casts. In addition, there were two other significant alterations in the kidney: vascular lesions involving the arterioles, and glomerular lesions. The arteriolar lesions were present in the afferent arterioles and the distal portions of the interlobular arteries. There was fibrinoid, eosinophilic, smudging necrosis of the vessel wall associated with varying degrees of intimal proliferation (Fig. 1*F* and *G*; Fig. 2*A*). In some vessels the intimal proliferation predominated and filled the lumen with concentric, reticulated cell layers presenting a typical onion-skin appearance (Fig. 2*B*). Foam cells were sometimes noted in the interstices of the layers. These lesions were not accompanied by inflammatory infiltration. The glomerular changes were widespread. Almost all glomeruli were ischemic. Epithelial cells were increased in number and prominence. The tufts were frequently fused, obliterated, or adherent to Bowman's capsule. The basement membrane of the capillaries was frequently swollen and presented a foamy appearance (Fig. 3*A* and *B*). Vacuolated cells were often seen in the tufts. Fat stains showed fat globules in these glomeruli as well as in many proximal convoluted tubules (Fig. 3*C* and *D*). With polarized light, some of the lipid was birefringent, and an occasional cross of polarization

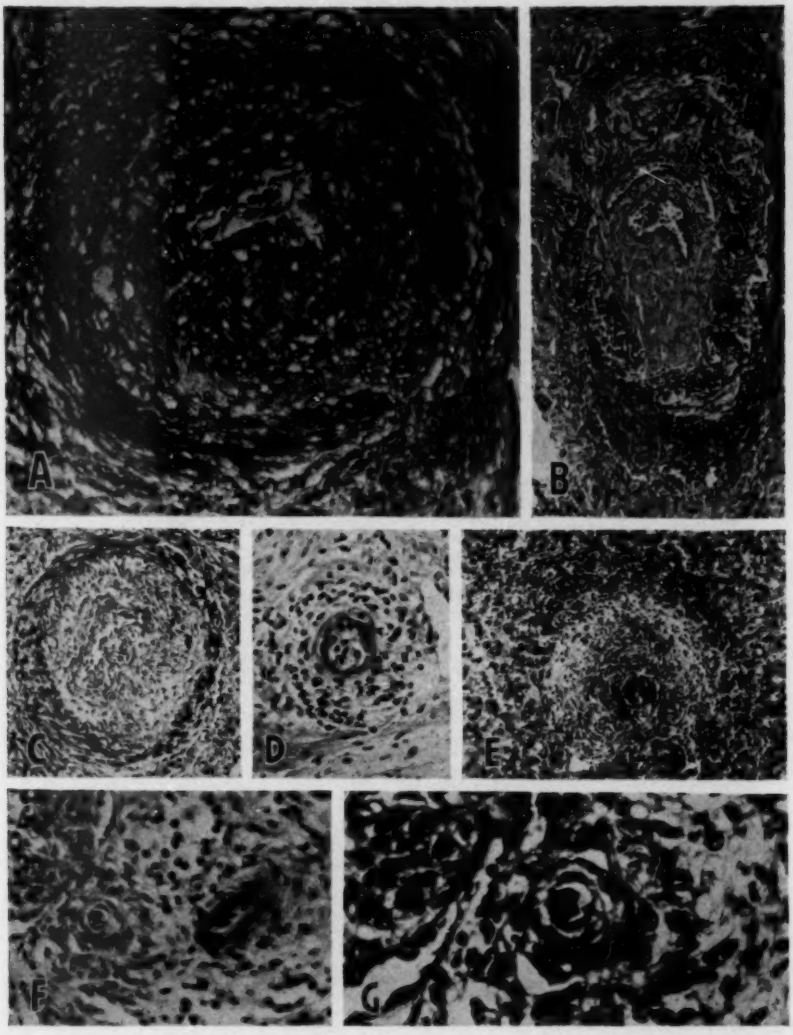


Fig. 1.—*A*, *B*, and *C*, arcuate arteries of kidney showing healing phase of periarteritis nodosa. Note disruption and partial destruction of internal elastic membrane, intimal proliferation, and adventitial fibrosis. *A*, Weigert; × 193.5. *B*, hematoxylin-eosin; × 96.5. *C*, hematoxylin-eosin; × 96.5.

*D*, arteriole of stomach showing acute phase of periarteritis. Note fibrinoid necrosis of the wall and periarterial inflammatory cell infiltration. Hematoxylin-eosin; ×193.5.

*E*, arteriole of liver showing acute phase of periarteritis. Note advanced fibrinoid necrosis of the wall and perivascular inflammatory cell infiltration. Hematoxylin-eosin; ×96.5.

*F* and *G*, afferent glomerular arterioles of kidney illustrating malignant nephrosclerosis. Note marked fibrinoid necrosis of the wall and absence of inflammatory cells. *F*, hematoxylin-eosin; ×193.5. *G*, hematoxylin-eosin; ×459.5.

(maltese cross) was observed (Fig. 3*E*). In the central nervous system there were organizing hemorrhages of an age consistent with the clinical history. Examination of a large number of sections revealed fibrinoid necrosis (smudging) in the walls of several arterioles, without perivascular or panvascular inflammation.

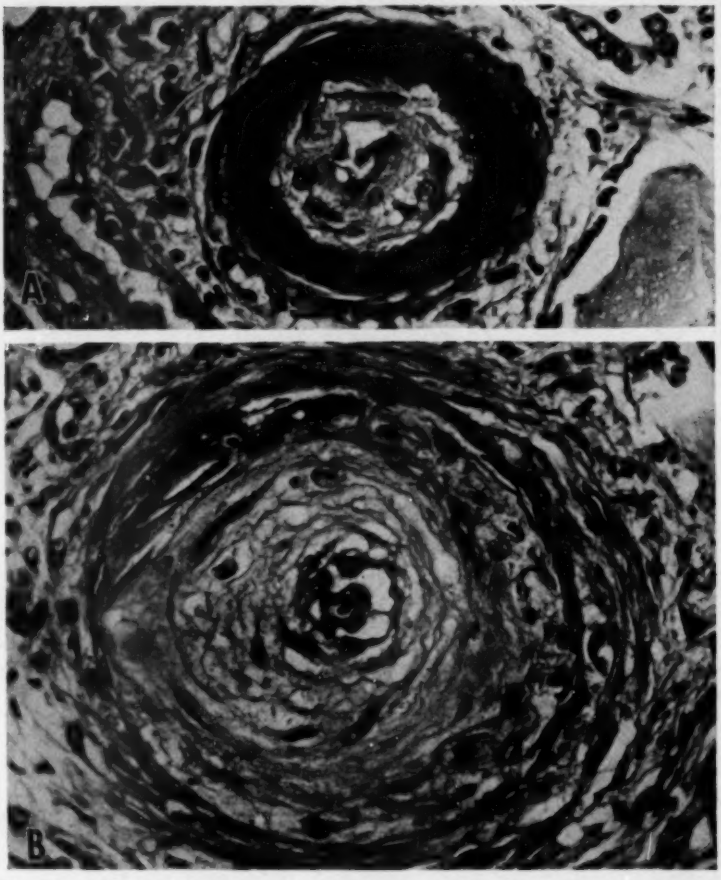


Fig. 2.—*A* and *B*, terminal portion of interlobular arteriole of kidney illustrating malignant nephrosclerosis. Note fibrinoid necrosis of the wall (most pronounced in *A*) and intimal proliferation (most pronounced in *B*); also, lipophages within the zone of intimal proliferation and absence of inflammatory cells. Hematoxylin-eosin; ×475.

In the pituitary there was a small focal collection of chromophobe cells intermingled with basophils and acidophils.

The areas where adrenal and kidney were grossly fused showed, for the most part, a distinct capsule between the two organs. There were, however, small areas where there was no distinct line of demarcation between kidney and adrenal. The cortex and the medulla of the adrenal were normal. The lung showed marked con-

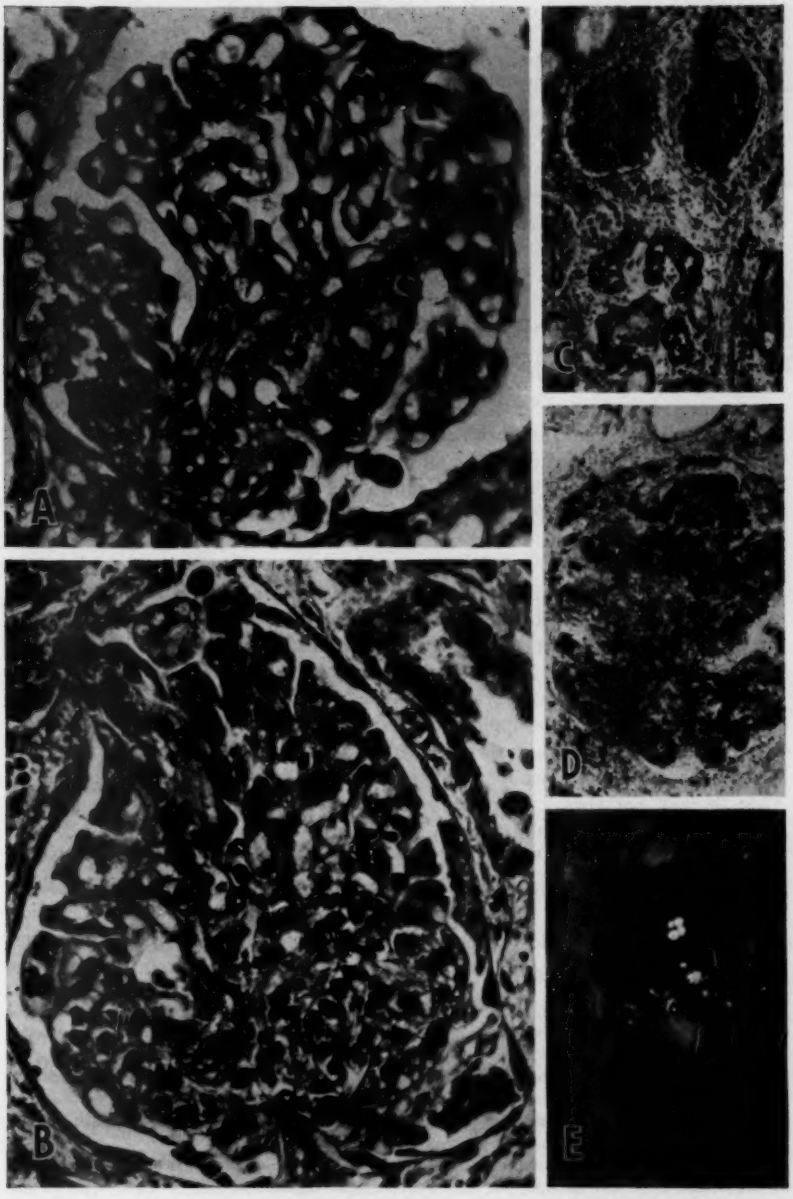


Fig. 3.—*A* and *B*, glomerulus of kidney showing membranous and proliferative glomerulonephritis, with increased cellularity, adhesions of tufts, thickened foamy basement membrane, and lipid-laden cells. Hematoxylin-eosin × 459.5.

*C* and *D*, fat in glomeruli and tubules. *C*, oil red O; × 96.5. *D*, oil red O; × 193.5.

*E*, tubule of kidney showing birefringent lipid material in the wall and a cross of polarization (maltese cross). Oil red O photographed with polarized light; ×459.5.

gestion and edema and areas of bronchopneumonia. The mitral verrucae were made up of swollen, elongated collagen fibers which merged with the connective tissue of the valve. This confirmed the gross impression of degenerative verrucal endocardiosis.[6]

*Final Diagnoses.*—Periarteritis nodosa involving heart, liver, pancreas, adrenals, kidneys, testes, gastrointestinal tract and striated muscle; malignant nephrosclerosis; membranous glomerulonephritis; cerebral hemorrhage; bronchopneumonia; adrenal-renal fusion; verrucal endocardiosis.

## COMMENT

There is ample histologic evidence that the periarteritis nodosa antedated the cortisone therapy and was the cause of the joint and muscle tenderness and of the fever. In addition, there was an acute membranous glomerulonephritis,[7] as well as a superimposed vascular lesion of the kidney which is typical of malignant nephrosclerosis.[8] Some authors have stressed the similarity between the vascular renal lesions of periarteritis nodosa and those of malignant nephrosclerosis.[9]

It is, however, possible to distinguish between these lesions. Allen [7] and Davson, Ball, and Platt [10] have presented several criteria which may be used in disentangling the vascular lesions which were described in the kidney in this case. In periarteritis nodosa (*a*) medium-sized and large arteries are involved, (*b*) inflammatory infiltration is constant (more pronounced in the acute phase) and (*c*) disruption and destruction of the internal elastic lamina are frequent. In malignant nephrosclerosis (*a*) only arterioles (afferent and distal portions of interlobular arteries) are involved, (*b*) there is no significant inflammatory infiltration and (*c*) there is no alteration of the internal elastic lamina.

These criteria cannot be applied to rigidly, for there may be some overlapping of lesions. Periarteritic changes are sometimes noted in arterioles, and malignant nephrosclerosis is occasionally accompanied by a discrete inflammatory exudate. Thus, it may not always be possible to identify a given single lesion, although typical lesions present little difficulty in differentiation (compare Fig. 1*A* to *E* with Fig. 2*A* and *B*). Allen,[7] in his discussion of the differential diagnoses of these two conditions, insists on the importance of a general appraisal of all lesions.

The arteriolar lesions of the central nervous system were considered typical of malignant sclerosis. However, few lesions were found, although many sections were examined. On theoretical grounds the cerebral hemorrhages may be

6. Allen, A. C., and Sirota, J. H.: Morphogenesis and Significance of Degenerative Verrucal Endocardiosis (Terminal Endocarditis, Endocarditis Simplex, Non-Bacterial Thrombotic Endocarditis), Am. J. Path. **20**:1025-1056, 1944.

7. Allen, A. C.: The Kidney: Medical and Surgical Diseases, New York, Grune & Stratton, Inc., 1951.

8. Klemperer, P., and Otani, S.: "Malignant Nephrosclerosis" (Fahr), Arch. Path. **11**:60-117 (Jan.) 1931.

9. (*a*) Fahr, T.: Maligne nephrosklerose und Periarteriitis nodosa, Deutsche med. Wchnschr. **67**:1223-1226, 1941. (*b*) Kountz, W. B.: Periarteritis Nodosa; Report of Case, Arch. Path. **10**:55-65 (July) 1930. (*c*) Grant, R. T.: Observations on Periarteritis Nodosa, Clin. Sc. **4**:245, 1939-1942. (*d*) Canny, A. J.: Unusual Renal Lesions Associated with Vascular Hypertension, M. J. Australia **2**:631-632, 1940.

10. Davson, J.; Ball, J., and Platt, R.: The Kidney in Periarteritis Nodosa, Quart. J. Med. **17**:175-202, 1948.

attributed either to periarteritis nodosa or to malignant sclerosis. The scanty histologic evidence available favors malignant sclerosis. Glomerulonephritis of necrotizing exudative or proliferative type may accompany either periarteritis nodosa [11] or malignant nephrosclerosis.[12] In this case, however, there was membranous glomerulonephritis with considerable deposition of fat within both tubules and glomeruli. The fat was present in unusually large amounts (Fig. 3*C*, *D* and *E*), and its presence was suspected before special stains were done. The onset of the glomerular lesions is undoubtedly related to the abrupt onset of urinary abnormalities (albuminuria, casts, and red blood cells) noted shortly after cessation of cortisone therapy.

The evolution of the blood urea nitrogen with its peak at 48 mg. and its slow regression to normal are difficult to explain. Judging from the microscopic aspect of the kidney one would have expected uremia with an elevation of blood urea nitrogen. The widespread acute periarteritic lesions in the liver may have been a factor in masking high blood urea nitrogen. Moreover, data concerning the terminal urea retention are not available inasmuch as blood urea nitrogen was last determined five days before death.

The adrenal-renal fusion probably has no clinical or pathogenic significance. It has been recorded in large routine autopsy series (in 8 of 5,000 cadavers in one series [13]). These cases were not accompanied with endocrine disturbances.

It is obviously difficult to determine with any degree of certainty the effects of cortisone in this case. In general, two possibilities may be considered:

(*a*) In this case malignant nephrosclerosis represented a form of evolution of periarteritis nodosa. Therefore, its occurrence immediately following cortisone therapy was fortuitous.

(*b*) There was a relation of cause and effect between the administration of cortisone and the advent of malignant nephrosclerosis.

Malignant nephrosclerosis does occasionally occur in the course of periarteritis nodosa. The incidence is difficult to determine. There have been reports of isolated cases of periarteritis nodosa associated with malignant nephrosclerosis.[14] Several large series of cases culled from the literature have been reviewed,[15] and hypertension has been found to be a common feature. Unfortunately, the type of hypertension is not always indicated, nor are sufficient data presented to enable an analysis of the cases. Reports of small series of cases, although statistically less significant, do provide important information. Harris, Lynch, and O'Hare [15a] found malignant hypertension in one of six cases of periarteritis nodosa; Spiegel [16] in two of 17 cases, and Grant [9c] in one of six cases. Thus, the incidence would seem

11. Allen.[7] Davson and others.[10]

12. Klemperer and Otani.[8] Allen.[7]

13. O'Crowley, C. R., and Martland, H. S.: Adrenal Heterotopia, Rests and So-Called Grawitz Tumor, J. Urol. **50**:756-768, 1943.

14. Mallory, T. B.: Cabot Case 22271, New England J. Med. **215**:41-43, 1936. Fahr.[9a]

15. (*a*) Harris, A. W.; Lynch, G. W., and O'Hare, J. P.: Periarteritis Nodosa, Arch. Int. Med. **63**:1163-1182 (June) 1939. (*b*) Strong, G. F.: Periarteritis Nodosa, Canad. M. A. J. **19**:534-546, 1928. (*c*) Curtis, A. C., and Coffey, R. M.: Periarteritis Nodosa: A Brief Review of the Literature and a Report of One Case, Ann. Int. Med. **7**:1345-1358, 1934.

16. Spiegel, R.: Clinical Aspects of Periarteritis Nodosa, Arch. Int. Med. **58**:993-1040 (Dec.) 1936.

to be small. Platt [17] in 1948 presented a careful analysis of 191 cases of severe hypertension and thus provided a somewhat different approach to this problem. In his group there were 20 cases of malignant hypertension, in two of which it was associated with periarteritis nodosa (one proved by autopsy). This confirms the relatively infrequent occurrence of malignant nephrosclerosis in the course of periarteritis nodosa.

Nevertheless, the possibility still obtains that in the case reported herein cortisone had no effect on the development of malignant hypertension. In considering the likelihood of an effect of cortisone, it should be stressed that cortisone was administered for a very short time and that the total amount given was very small (600 mg.). Therefore, it would seem that a trigger mechanism operated in this case, setting off a process which culminated in the vascular lesions of malignant nephrosclerosis. This is not as improbable as it may seem at first thought. The beneficial effects following cortisone therapy are often reported as immediate and dramatic, frequently after the first two or three doses.[18] Therefore, it is not unreasonable to anticipate that the untoward effects of cortisone may be precipitated, under certain circumstances, by equally small doses and in a similar sudden manner. Experimentally, both periarteritis and what is described as malignant nephrosclerosis may be produced by administration of mineralocorticoids.[1] Therefore, both may be the result of an adaptive mechanism or of a hormonal imbalance. Glucocorticoids (cortisone) have a pronounced inhibitory effect on the peripheral vasotoxic effect of mineralocorticoids.[1] However, Selye [1] has stated that in some animals malignant nephrosclerosis has developed following administration of cortisone. This paradoxical effect is not clearly explained. Apparently, it may be due to the presence of small amounts of mineralocorticoids in cortisone. The toxic effect of corticoids is enhanced by previous renal damage.[1]

The periarteritic lesions in this case clearly antedated the period of cortisone therapy. It is reasonable to assume that there were extensive renal vascular lesions and ischemia at the time of cortisone administration. This state of renal ischemia may have been one of the factors which determined the toxic effect of cortisone and the development of malignant nephrosclerosis and membranous glomerulonephritis.

## CONCLUSIONS

In a case of periarteritis nodosa, cortisone therapy was immediately followed by fatal malignant hypertension. At autopsy there was periarteritis nodosa of many organs, including the kidney. These lesions were in acute, healing, and healed phases. There were also membranous glomerulonephritis and lesions typical of malignant nephrosclerosis. The renal vascular lesions of malignant nephrosclerosis may be distinguished from vascular lesions attributable to periarteritis nodosa. It is suggested that a relation of cause and effect may exist between the administration of cortisone and the occurrence of malignant nephrosclerosis in this case.

Dr. Arthur C. Allen contributed invaluable advice in the interpretation of many aspects of this problem.

The photomicrographs used in this article were made by the Medical Illustration Service of the Veterans Administration Hospital, Bronx, N. Y.

17. Platt, R.: Severe Hypertension in Young Persons, Quart. J. Med. **17**:83-93, 1948.
18. Shick and others.[4a] Goldman and others.[4b] Sharnoff and others.[5]

# QUANTITATIVE EXFOLIATIVE CYTOLOGY

## The Effect of Diethylstilbestrol on the Glycogen Content of the Epithelial Cells in Urinary Sediment and Cervical Smears in Pregnancy

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AND
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THE GLYCOGEN content of epithelial cells in urine-sediment smears and in cervical smears representing normal pregnant women and pregnant women receiving diethylstilbestrol was studied by techniques described previously.[1] Differential counting of cervical smears had shown a higher percentage of glycogen-containing epithelial cells from pregnant than from nonpregnant women.[1a] In a later study, in which urine-sediment smears were counted,[1b] no difference was found in the percentage of glycogen-containing epithelial cells from pregnant as compared with nonpregnant women.

In the present study pregnant women receiving diethylstilbestrol had a lower percentage of glycogen-containing cells in cervical smears and a somewhat lower percentage of such cells in urine-sediment smears than did normal pregnant women. This difference was less marked in the urinary than in the cervical smears.

### MATERIALS AND METHODS

Urine and cervical material were obtained from 16 normal pregnant women and from 14 pregnant women receiving diethylstilbestrol, ranging from the fourth to the eighth month of gestation. Twelve of the pregnant women had hypertensive cardiovascular disease (essential hypertension), one was a diabetic under control, and the remaining patient had a history of repeated abortion. These women received from 30 to 105 mg. of diethylstilbestrol daily, according to the schedule of Smith.[2]

The cervical material was obtained by superficial scraping and the smears were immediately fixed in ether-alcohol. The urine specimens were centrifuged; smears were made of the sediment and fixed in ether-alcohol. All smears were stained by the periodic acid technique.[3] Half of the smears were digested with saliva before staining, to remove glycogen,[4] since certain sub-

From the Departments of Pathology and Obstetrics and Gynecology, Emory University School of Medicine, and Grady Memorial Hospital.

1. (*a*) Foraker, A. G., and Brawner, D. L.: Quantitative Exfoliative Cytology: Differential Counting of Cervical Smears Stained for Glycogen in Cases of Pregnant and Nonpregnant Women, A. M. A. Arch. Path. **51**:201 (Feb.) 1951. (*b*) Foraker, A. G., and Keye, J. D., Jr.: Quantitative Exfoliative Cytology: An Evaluation of the Glycogen Content of Exfoliated Epithelial Cells in Urine Sediment from Pregnant and Nonpregnant Women, ibid. **51**:351 (March) 1951.

2. Smith, O. W.: Am. J. Obst. & Gynec. **56**:821, 1948.

3. Lillie, R. D.: Histopathologic Technique, Philadelphia, The Blakiston Company, 1948, p. 145.

4. Carpenter, A.; Polonsky, B., and Menten, M. L.: Arch. Path. **51**:480, 1951. Bourne, G.: Cytology and Cell Physiology, Oxford, Oxford University Press, 1942, p. 23. Glick, D.: Techniques of Histo- and Cytochemistry, New York, Interscience Publishers, Inc., 1949, p. 48.

stances other than glycogen give a positive reaction with this staining technique.[1] All differential counts were performed as "unknowns" by the same person (A. G. F.). More than 2,000 cells were counted on each digested and undigested cervical smear. More than 1,000 cells were counted on each digested and undigested urine-sediment smear. In this study, more than 6,000 epithelial cells from each patient were counted. No attempt was made to identify specific epithelial-cell types or to record cytologic detail. In all smears the cells staining positively (purple) were readily differentiated from those failing to take the stain.

The findings from the cervical smears are summarized in Table 1. In the undigested smears (A) the percentage of positively staining cells represents the cells containing glycogen and other substances reacting to the periodic acid routine. Since salivary amylase is presumed to break down glycogen,[5] the percentage of

TABLE 1.—*Differential Counts of Cervical Epithelial Cells Staining Positively with the Periodic Acid Routine from Sixteen Normal Pregnant Women and Fourteen Pregnant Women Receiving Diethylstilbestrol*

| | Mean Percentages (and Standard Errors) of Positively Staining Cells | | | |
|---|---|---|---|---|
| | Normal Pregnant Women (16) | Diethylstilbestrol-Treated Pregnant Women (14) | Significance Ratio ($t$) | $p$ |
| A. Before saliva digestion of smears.. | 57.44 ± 3.07 | 35.86 ± 5.07 | 3.65 | $< 0.01$ |
| B. After saliva digestion..... | 9.41 ± 2.72 | 7.92 ± 1.91 | 0.45 | $> 0.50$ |
| A—B. Glycogen-containing cells..... | 48.03 ± 3.83 | 27.94 ± 5.08 | 3.18 | $< 0.01$ |

TABLE 2.—*Differential Counts of Epithelial Cells in Urine Sediment Staining Positively with the Periodic Acid Routine from Sixteen Normal Pregnant Women and Fourteen Pregnant Women Receiving Diethylstilbestrol*

| | Mean Percentages (and Standard Errors) of Positively Staining Cells | | | |
|---|---|---|---|---|
| | Normal Pregnant Women (16) | Diethylstilbestrol-Treated Pregnant Women (14) | Significance Ratio ($t$) | $p$ |
| C. Before saliva digestion of smears.. | 59.25 ± 3.33 | 50.42 ± 3.08 | 1.95 | $0.10 > p > 0.05$ |
| D. After saliva digestion..... | 2.45 ± 0.70 | 3.24 ± 1.52 | 0.47 | $> 0.50$ |
| C—D. Glycogen-containing cells..... | 56.80 ± 3.09 | 47.18 ± 3.39 | 2.10 | $0.05 > p > 0.02$ |

positively staining cells in the saliva-digested smears (B) should represent the cells containing substances other than glycogen which react to the periodic acid routine. Subtracting the percentage of positively staining cells in the saliva-digested smears (B) from the percentage of positively staining cells in the undigested smears (A) should reveal the percentage of cells which owed their staining reaction largely to glycogen (A — B). For convenience these will be termed glycogen-containing cells. The normal pregnant women had a higher mean percentage of glycogen-containing cells (A — B) and of positively staining cells in the undigested smears (A) than did the diethylstilbestrol-treated women. There was no striking difference in the percentage of positively staining cells in the saliva-digested smears (B).

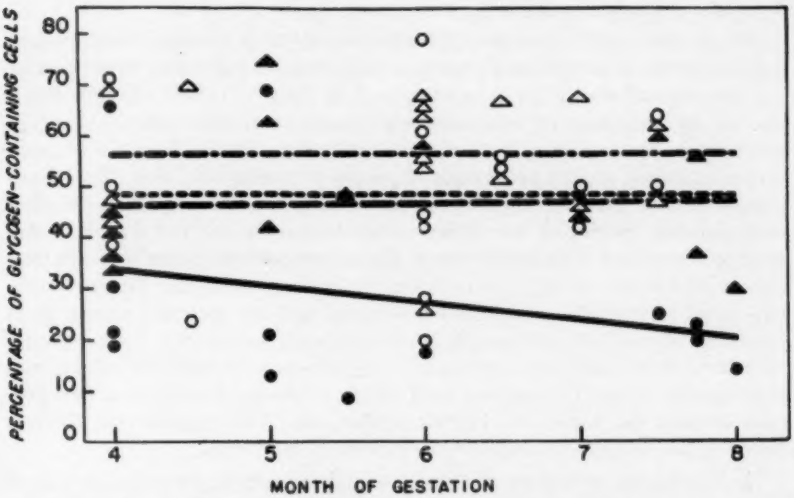
Computations were similarly applied to the urine-sediment smears, the results being summarized in Table 2. Smears from the normal pregnant women showed a somewhat higher mean percentage of glycogen-containing cells (C — D) and of

5. Bondy, P. K.; Sheldon, W. H., and Evans, L. D.: J. Clin. Invest. **28**:1216, 1949.

cells staining positively in the undigested smears (C) than did the smears from the diethylstilbestrol-treated women. The difference was less striking than that obtained in the cervical smears. Again there was little difference in the percentage of positively staining cells in the saliva-digested smears (D).

There was no evidence of correlation between high or low percentage of glycogen-containing cells in urine-sediment and cervical smears from the same patient among either normal ($r = 0.1467$) or diethylstilbestrol-treated ($r = -0.0693$) women.[6b]

Women in later stages of gestation received higher doses of diethylstilbestrol.[2] In our series the percentage of glycogen-containing cells in cervical smears declined with the increase of diethylstilbestrol dose, as shown in the chart. Percentage of glycogen-containing cells computed against month of gestation resulted in a regres-




Percentages of glycogen-containing epithelial cells in urine-sediment and cervical smears from 16 normal pregnant women and 14 diethylstilbestrol-treated pregnant women, by month of gestation (with regression lines). Cervical smears: The percentages for normal pregnant women are represented by white circles and a broken line (- - - -); those for diethylstilbestrol-treated women, by black circles and a solid line (———). Urine-sediment smears: The percentages for normal pregnant women are represented by white triangles and a dash-dot line (—.—.); those for diethylstilbestrol-treated women, by black triangles and a dash line (— — —).

sion rate ($b$) of $-3.415$.[6c] There was no apparent change in the percentage of glycogen-containing cells in cervical smears from the normal pregnant women ($b = -0.0142$) in later stages of gestation. The percentage of glycogen-containing cells in urine sediment showed little evidence of change with the month of gestation in either normal ($b = 0.0886$) or diethylstilbestrol-treated ($b = 0.1577$) women.

Smears from the diabetic patient conformed rather closely to the mean values for those from other diethylstilbestrol-treated women, as to percentage of glycogen-

6. Snedecor, G. W.: Statistical Methods Applied to Experiments in Agriculture and Biology, ed. 4, Ames, Iowa, The Iowa State College Press, 1948, (*a*) p. 65; (*b*) 139; (*c*) 137.

containing cells in both cervical smears (A — B = 29.94%) and urine-sediment smears (C — D = 40.29%).

## COMMENT

It is difficult to decide whether the changes observed were related to diethylstilbestrol treatment or to the underlying disorders that led to the administration of the estrogen. There is little reason to believe that hypertensive cardiovascular disease might be responsible for the present findings. The only factor common to the treated women was the diethylstilbestrol. It seems likely that diethylstilbestrol was responsible for most of the findings in this series.

Several investigators [7] have reported that the glycogen in vaginal cells varied with the estrogenic level or with estrogen therapy. Ayre [8] has stated that the vaginal smear accompanying an induced elevation of the estrogen level ("hyperestrinism") is usually glycopenic in character.

In our first study [1a] there was little difference in the percentage of cells staining positively with the periodic acid routine in undigested cervical smears from pregnant and nonpregnant women (corresponding to A in Table 1). There was little difference in the percentage of cells containing glycogen and other substances (e. g., mucoproteins [9]) reacting to the periodic acid routine. Smears from the pregnant women contained smaller percentages of positively staining cells after saliva digestion (e. g., B in Table 1). These smears had fewer cells containing substances other than glycogen reacting to the periodic acid routine. It was this difference that resulted in a computed higher percentage of glycogen-containing cells in smears from the pregnant women (e. g., A — B, Table 1). In our present study little difference was found between the diethylstilbestrol-treated and the untreated women as to percentage of positively staining cells in saliva-digested smears (B). The two groups owed their differentiation to a variation in the percentage of positively staining cells in undigested smears (A) and not indirectly to a variation in content of mucoproteins or other substances (B) in their cervical cells. This suggests that different physiological mechanisms produced the differences established:

1. The higher percentage of glycogen-containing cells in cervical smears from pregnant women as compared with nonpregnant women found in our first study.[1a]
2. The higher percentage of glycogen-containing cells in cervical smears from normal pregnant women as compared with pregnant women receiving diethylstilbestrol in the present study.

The absence of apparent change with stage of gestation in the percentage of glycogen-containing cells in urine sediment from the normal pregnant women is in accord with our previous findings.[1b]

No attempt is made at present to apply our results in a consideration of the general rationale of diethylstilbestrol therapy for certain disorders of pregnancy.[2]

7. Mack, H. C.: Am. J. Obst. & Gynec. **45**:402, 1943. Willson, J. R., and Goforth, M. L.: J. Clin. Endocrinol. **2**:223, 1942. Siddall, R. S.: Am. J. Obst. & Gynec. **47**:260, 1944.

8. Ayre, W. B.: J. Clin. Endocrinol. **11**:103, 1951.

9. McManus, J. F. A.: Am. J. Path. **24**:643, 1948.

**SUMMARY**

Differential counts of urine-sediment and cervical smears stained by the periodic acid routine from 16 normal pregnant women and 14 pregnant women receiving diethylstilbestrol showed:

1. A lower percentage of glycogen-containing epithelial cells in cervical smears from the diethylstilbestrol-treated women.
2. A less well defined reduction in the percentage of glycogen-containing epithelial cells in urine-sediment smears from the diethylstilbestrol-treated women.
3. No evidence of correlation between the percentages of glycogen-containing cells in urine-sediment and cervical smears in individual cases.
4. A reduced percentage of glycogen-containing cells in cervical smears from the pregnant women receiving diethylstilbestrol in later stages of gestation (higher doses) as compared with diethylstilbestrol-treated women in earlier stages of gestation and with normal pregnant women in comparable stages of gestation.
5. No apparent change in percentage of glycogen-containing cells in cervical smears from normal pregnant women with stage of gestation (from the fourth to the eighth month).
6. No apparent change in percentage of glycogen-containing cells in urine-sediment smears from normal or diethylstilbestrol-treated women with stage of gestation (from the fourth to the eighth month).

# DEATHS FROM RAPID ANOXIA

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FOR MANY years, rapid deaths of medicolegal importance have been classified into three groups according to the supposed mechanisms of death, viz., coma, syncope, and asphyxia. Although these terms may describe terminal dominant symptoms, they do not provide an index of the mechanism of death, and the clinical conditions which they represent cannot be recognized at necropsy by any specific pathological changes. Many rapid deaths of medicolegal importance are fundamentally anoxic deaths, and for practical purposes such deaths may be classified by the manner in which the anoxia is *initiated*.

## CAUSES OF RAPID ANOXIC DEATHS

A. Deaths initiated by defective oxygenation of the blood in the lungs (anoxic anoxia):

1. From breathing in a vitiated atmosphere. These deaths are caused when (*a*) oxygen is displaced from the atmosphere by inert gases, e. g., the fumes in wells and vats; or (*b*) oxygen is combined with gases in the atmosphere, e. g., "sewer gas."

2. From mechanical developments that interfere with the passage of air into or down the respiratory tract. Such obstruction may be caused either (*a*) by closure of the external respiratory orifices, e. g., in smothering and overlaying, or (*b*) by obstruction of the air passages, e. g., in drowning, in foreign body impaction and in *certain* cases of throttling, strangulation, and hanging.

3. From external compression on the chest and abdominal walls interfering with respiratory movements, e. g., falls of earth.

4. From a primary cessation of respiratory movements through paralysis of the respiratory center, e. g., in certain cases of electrical injury and in certain forms of acute poisoning.

B. Deaths initiated by a reduction of the oxygen-carrying capacity of the blood (anemic anoxia). This form of death occurs in cases of acute carbon monoxide poisoning and cases of acute massive hemorrhage.

C. Deaths initiated by a depression of the oxidative processes in the tissues (histotoxic anoxia). This form of death is seen in cases of acute cyanide poisoning.

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From the Union Health Department, Durban (Dr. Gordon, senior government pathologist) and the Union Health Department, Capetown (Dr. Turner, senior government pathologist, professor of medical jurisprudence, University of Capetown).

### MECHANISM OF FAILURE IN RAPID ANOXIC DEATHS

The mechanism of failure in rapid anoxic deaths cannot readily be determined by ordinary clinical methods, and such observations alone do not necessarily afford reliable evidence of the nature of the failure. With human beings it is usually impossible to obtain continuous records of the sequence of physiological events. Numerous animal experiments, however, have been undertaken to determine the main cardiorespiratory and biochemical changes which occur in various types of rapid anoxic death. In most of these experiments, the investigations have been confined to a single type of anoxic death and often only one physiological system has been studied at a time. In order to obtain comprehensive information about the process of anoxic deaths, Swann [1] (1948) and Swann and Brucer [2] (1949) devised a series of experiments on unanesthetized dogs. They investigated the following types of anoxic deaths: obstructive asphyxia; drowning in fresh water; sea water drowning; carbon monoxide poisoning; exsanguination, and deaths caused by breathing gas mixtures low in oxygen, i. e., while breathing pure nitrogen, while breathing 2.43 per cent oxygen in nitrogen, and while rebreathing through soda lime. In each experiment simultaneous observations were made of systolic and diastolic arterial blood pressures, pressure in the inferior vena cava, heart rate, heart sounds, electrocardiograms, intrathoracic pressure, pulmonary ventilation, respiratory rate, blood acidity, blood oxygen, blood carbon dioxide, blood lactic acid, hemoglobin, and plasma proteins.

These experiments showed that there were great individual differences in the times of survival of dogs subjected to various types of anoxic death. In fulminating anoxia caused by inhalation of pure nitrogen the respiratory cessation occurred after the cardiac cessation, but in the majority of the deaths the respiratory cessation occurred at approximately the same time as the cardiac cessation. The process of circulatory failure was invariably rapid, cardiac cessation occurring three to five minutes after the oxygen saturation fell to 10 per cent or less. The animals often showed low diastolic pressures, but the systolic pressures remained elevated until death was imminent. At the time of death the systolic pressures declined sharply. Because of this sharp fall of systolic pressure, Swann [3] believes that the final circulatory failure in anoxic deaths is determined by myocardial anoxia rather than by sudden peripheral vasodilatation.

In these experiments, regardless of the type of death, the blood carbon dioxide content never exceeded 60 volumes per cent. In the cases of obstructive asphyxia and drowning there was an initial rise in carbon dioxide content, but this fell with the concomitant acidosis. A respiratory alkalosis often occurred in the early phases of the anoxia, but as death became imminent, the blood pH values usually fell to a relatively low level. In carbon monoxide poisoning and exsanguination the electrocardiographic changes showed special features of differentiation, but in all the other types of anoxic death the electrocardiograms showed the same constant

1. Swann, H. G.: Studies in Resuscitation, USAF Air Materiel Command Memorandum Report MCREXD series 696-79 G., 1948.

2. Swann, H. G., and Brucer, M.: The Cardiorespiratory and Biochemical Events During Rapid Anoxic Death, Texas Rep. Biol. & Med. **7**:511, 1949.

3. Swann, H. G.: Personal communications to the authors, 1950.

features. The terminal events in drowning were more complicated than in obstructive asphyxia because of the passage of electrolytes between the fluid in the alveoli and the blood plasma.

Apart from the electrocardiographic changes in carbon monoxide poisoning and exsanguination and the special features in drowning, the pattern of cardiorespiratory and biochemical changes was essentially similar in all the forms of rapid anoxic death that were studied. Although these experiments were carried out on dogs, Swann [3] (1950) believes that man reacts in a similar manner.

Swann and Brucer [2] did not record detailed morbid anatomical changes, but Swann [3] (1950) states that with the exception of the cases of drowning he did not observe any frank changes in the viscera which served to distinguish the different forms of anoxic death.

### THE PATHOLOGY OF RAPID ANOXIC DEATHS

During the process of death from rapid anoxia, widespread tissue injury results from the lack of oxygen. As the endothelium of the capillaries is particularly susceptible to anoxia, the injury of this tissue results in dilatation of the capillaries. This is followed by stasis of blood in the dilated capillaries and in the venules, which accounts for the capillovenous engorgement which is a major feature of the general pathological findings in rapid anoxic deaths.

Injury of the capillary endothelium is often accompanied with petechial hemorrhages in the tissues. These hemorrhages may be found in the substance of viscera, but they are most readily observed in the serous membranes, particularly in the visceral pleurae and the visceral pericardium. Although petechial hemorrhages are not found invariably in rapid anoxic deaths, they occur in the majority of such deaths.

Anoxic injury of the capillaries may also result in increased capillary permeability with transudation of plasma into the tissue spaces. In the early period after such injury, the transudation is followed by increased lymph drainage, and edema does not develop as long as this drainage is adequate. Equilibrium can be maintained for a relatively short period only, and if the anoxic process continues, edema of the tissues develops. Edema of the lungs may be seen, but generalized edema is not a prominent feature of the postmortem aspects. Slight nonspecific parenchymatous degenerative changes may be found in rapid anoxic deaths. The rapidity with which such changes develop in the liver has been shown by Gillman and Gillman [4] (1948).

Capillovenous congestion of the viscera and petechial hemorrhages of the serous membranes are nonspecific general pathological changes which occur in all forms of rapid anoxic deaths. These changes are also seen in deaths from secondary shock and in many deaths from natural causes. Edema of the viscera and effusions into serous cavities are commonly described in deaths from secondary shock, and the extent and the degree of parenchymatous degenerative changes are relatively more impressive than in rapid anoxic deaths. The essential similarity in the post-

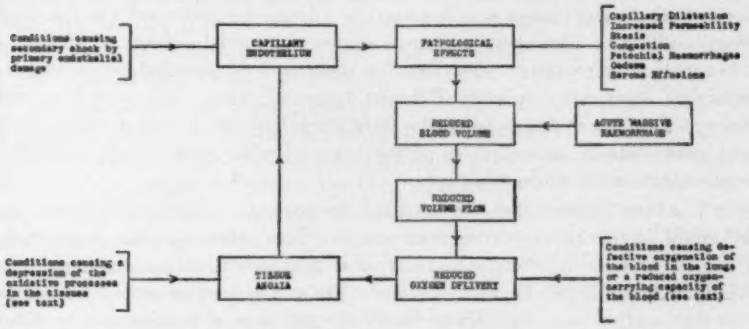
4. Gillman, J., and Gillman, T.: Anoxia and the Liver with a Special Reference to Shock and Chronic Malnutrition, South Africa J. M. Sc. **13**:11, 1948.

mortem findings of rapid anoxic deaths and deaths from secondary shock has been emphasized by Moon [5] (1938) and Moritz [6] (1942).

The accompanying diagram, which has been adapted from the work of Moon [7] (1942), sets out the relationship between the various conditions causing rapid anoxia and secondary shock. The diagram illustrates how rapid anoxia, initiated in various ways, gives rise to the same vicious cycle of circulatory failure that occurs in secondary shock.

## ASPHYXIA AS A PATHOLOGICAL ENTITY

The concept that asphyxia is a pathological entity which can be recognized by certain specific pathological changes has led to considerable confusion in the literature on forensic medicine. Some authors use the term "asphyxia" synonymously with "anoxia." It is used by others to describe forms of death which are initiated by defective oxygenation of the blood in the lungs (anoxic anoxia). There are others who restrict its use to that group of deaths which result from a mechanical


Conditions causing secondary shock by primary endothelial damage
CAPILLARY ENDOTHELIUM
PATHOLOGICAL EFFECTS
Capillary Dilatation
Increased Permeability
Stasis
Congestion
Petechial Haemorrhages
Oedema
Serous Effusions
REDUCED BLOOD VOLUME
ACUTE MASSIVE HAEMORRHAGE
REDUCED VOLUME FLOW
Conditions causing a depression of the oxidative processes in the tissues (see text)
TISSUE ANOXIA
REDUCED OXYGEN DELIVERY
Conditions causing defective oxygenation of the blood in the lungs or a reduced oxygen-carrying capacity of the blood (see text)

The vicious cycle of the circulatory failure in rapid anoxia and secondary shock as adapted from Moon.[7]

interference with respiration. It is in the latter sense that the term is usually employed and understood in medicolegal practice.

It has been claimed on pathological grounds that visceral congestion, petechial hemorrhages, cyanosis, a condition of postmortem fluidity of the blood and cardiac dilatation are pathognomonic signs of asphyxia. Certain biochemical changes have also been regarded as being characteristic of this condition.

## VISCERAL CONGESTION

Capillovenous congestion of the viscera occurs as a general pathological change in all forms of rapid anoxic death, in deaths from secondary shock and in many deaths from natural causes.

5. Moon, V. H.: Shock and Related Capillary Phenomena, London, Oxford University Press, 1938, p. 211.

6. Moritz, A. R.: The Pathology of Trauma, Philadelphia, Lea & Febiger, 1942, p. 129.

7. Moon, V. H.: Shock: Its Dynamics, Occurrence and Management, Philadelphia, Lea & Febiger, 1942, p. 208.

Visceral congestion is usually prominent in deaths from mechanical interference with respiration. Swann and Brucer [2] have stated that man probably survives for several minutes in deaths from mechanical interference with respiration, and the work of Moon suggests that the intensity of the congestive changes in this form of death depends on the period of such survival rather than on any special mechanism of death. The intensity of the congestive changes, however, does not serve to differentiate such deaths, as similar changes are observed in many other forms of death.

### PETECHIAL HEMORRHAGES

Petechial hemorrhages in the visceral pleurae and visceral pericardium are often referred to as "Tardieu spots," as they were originally described by Tardieu in a series of papers published during the last century. Tardieu consistently found these hemorrhages in deaths from mechanical interference with respiration and regarded them as a pathognomonic sign of such deaths (Tardieu,[8] 1866). Ogston [9] (1868) recorded that soon after Tardieu published his original papers, his claim that petechial hemorrhages in the visceral pleurae and visceral pericardium were a specific pathological change was contested by Liman, the celebrated German medicolegal authority. The views of Liman receive substantial support at present, as pathologists have repeatedly described the occurrence of petechial hemorrhages in the serous membranes in many different forms of death. We have frequently observed such hemorrhages in deaths from secondary shock, and in many deaths from natural causes, as well as in all the forms of rapid anoxic death included in the classification set out in this paper.

It has been claimed that the petechial hemorrhages observed in deaths from mechanical interference with respiration differ from other petechial hemorrhages in respect to their number, size, color, and distribution. This claim has been investigated by Duncan-Taylor [10] (1950). The preliminary results of her studies show that on this basis the various forms of rapid anoxic death cannot be differentiated, either one from another, or from deaths due to secondary shock or natural causes.

### CYANOSIS

Cyanosis depends on an increase in the amount of reduced hemoglobin in the blood and may appear in any form of death in which the proportion of oxyhemoglobin in the blood is sufficiently diminished. Cyanosis is a common postmortem finding and is not confined to those deaths which are initiated by anoxic anoxia or which are caused by a mechanical interference with respiration.

### POSTMORTEM FLUIDITY OF THE BLOOD

When a postmortem examination is undertaken shortly after death, the blood is usually fluid, and it undergoes spontaneous coagulation when removed from the heart and vessels. If, however, the examination is undertaken a few hours after

8. Tardieu, H.: Á l'histoire de la mort par suffocation, Ann. d'hyg. publ. et de méd. légale **2**:357, 1866.

9. Ogston, F.: On Punctiform Ecchymoses in the Interior of the Body as a Proof of Death by Suffocation, Brit. M. J. **2**:332, 1868.

10. Duncan-Taylor, J. E.: Personal communication to the authors, 1950.

death, the blood may be clotted, or completely fluid and incoagulable, or partly clotted and partly fluid. In most deaths caused by mechanical interference with respiration, the blood is fluid and incoagulable, but this condition of the blood is not confined to such deaths.

Mole [11] (1948) studied the factors which are responsible for postmortem fluidity of the blood. He demonstrated the presence of a fibrinolysin in more than 90% of samples of fluid and incoagulable blood. This lysin is active only while thrombi are being formed, and Mole considers that the lysin is adsorbed on the thrombi and is released into solution when the fibrin is lysed. For this reason, a given concentration of fibrinolysin is more likely to liquefy a clot when the process of coagulation is slow. Under certain conditions the fibrinolysin may be so active that fibrin is destroyed as rapidly as it is produced, and postmortem thrombi never develop in the vessels. In other cases, thrombi are formed, but they undergo lysis.

In deaths from any cause, the condition of the blood at necropsy depends on the concentration of fibrinolysin and the rate of intravascular coagulation after death. When the fibrinolysin is active and the rate of coagulation is slow, the blood is likely to be fluid. When the fibrinolysin is less active and the rate of coagulation is rapid, postmortem thrombi are likely to form in the blood.

Mole has shown that in many deaths, from a wide variety of causes, fibrinolysin is produced and the blood is found to be fluid at necropsy. Following the suggestion of Macfarlane and Biggs [12] (1946), he stated that production of fibrinolysin might be part of the nonspecific general reaction to "injury" which characterizes Selye's "alarm reaction." Selye [13] (1949) has defined the "alarm reaction" as "the sum of all non-specific systemic phenomena due to sudden exposure to stimuli to which the organism is quantitatively or qualitatively not adapted." These considerations have led Mole to emphasize that postmortem fluidity of the blood is not characteristic of any special cause or mechanism of death.

## CARDIAC DILATATION

Cardiac dilatation has been described as one of the characteristic signs of asphyxia. Cardiac dilatation is essentially a clinical concept, and the use of the term in pathology for the purposes of morbid-anatomical description is restricted. According to Anderson [14] (1948), true cardiac dilatation can be recognized at necropsy only when it occurs in association with certain forms of cardiac hypertrophy.

In ascribing a special significance to cardiac dilatation in asphyxial deaths, it would appear that distention of the chambers of the heart has been regarded as evidence of cardiac dilatation. Distention of the auricles and the ventricles is a common postmortem phenomenon and may result from secondary muscular flaccidity. It has no significance as a specific pathological change.

11. Mole, R. H.: Fibrinolysin and the Fluidity of the Blood Post-Mortem, J. Path. & Bact. **60**:413, 1948.

12. Macfarlane, R. G., and Biggs, R.: Observations on Fibrinolysis, Lancet **2**:862, 1946.

13. Selye, H.: The General Adaptation Syndrome and the Diseases of Adaptation, Practitioner **163**:393, 1949.

14. Anderson, W. A. D.: Pathology, St. Louis, The C. V. Mosby Company, 1948, p. 533.

### BIOCHEMICAL CHANGES

The work of Swann and Brucer [2] shows that estimations of the oxygen content, the carbon dioxide content, and the hydrogen ion concentrations of agonal blood are of no value in differentiating obstructive asphyxial deaths from other forms of rapid anoxic deaths. The claim that hyperglycemia is characteristic of deaths from mechanical interference with respiration has been thoroughly investigated by Tonge and Wannan [15] (1949). These authors have shown that an estimation of the postmortem blood sugar is of no value in providing corroborative evidence of this condition. In a series of 145 cases, agonal hyperglycemia was found in 2 only of 23 cases of asphyxial deaths. Tonge and Wannan emphasized that an approximation to the agonal blood sugar level can be obtained only if the blood is taken from the left ventricle and if the true glucose value is estimated by yeast fermentation methods.

Except in cases of drowning, postmortem chemical examinations are of no greater value than morphological studies in differentiating so-called asphyxial deaths from other forms of death.

### COMMENT

In all forms of death the necropsy observations may be divided into two groups. The first group comprises those of general pathological changes, such as visceral congestion and petechial hemorrhages. These nonspecific changes are always present in a greater or less degree in rapid anoxic deaths as well as in deaths from secondary shock and natural causes. The second group consists of the pathological observations which are dependent on the type of death, e. g., those of local injuries to the neck in throttling and of the color of the blood in acute carbon monoxide poisoning. Such changes are best described as special pathological changes. As indicated in a previous paper by one of us (Gordon,[16] 1944) it appears that a sufficiently clear distinction is not always made between these two groups of postmortem observations. Consequently, nonspecific general pathological changes have been invested with the significance of special pathological changes. In this way asphyxia has come to be regarded as a pathological entity.

The records of certain criminal trials in South Africa show that penalties, including death sentences, were inflicted where medical testimony based on the view that asphyxia is a pathological entity was a major factor in securing the convictions. In these cases the deaths were ascribed to asphyxia (in the sense of a mechanical interference with respiration) because of the necropsy findings of visceral congestion, petechial hemorrhages, cyanosis, and postmortem fluidity of the blood alone.

The errors which arise when such findings are accepted as pathognomonic of asphyxia are not confined to criminal cases. Every year many infants who appear to be in good health are found dead in their cribs, cots and perambulators. Such deaths are usually ascribed to "accidental mechanical suffocation." Werne and Garrow [17] (1947) investigated the deaths of 167 infants belonging to the group

15. Tonge, J. I., and Wannan, J. S.: The Post-Mortem Blood Sugar, M. J. Australia **1**: 439, 1949.

16. Gordon, I.: A Classification of Deaths of Medico-Legal Importance, Brit. M. J. **2**:337, 1944.

17. Werne, J., and Garrow, I.: Sudden Deaths of Infants Allegedly Due to Mechanical Suffocation, Am. J. Pub. Health **37**:675, 1947.

which are ordinarily certified in this manner. In 124 of the 167 cases the cause of death could not be determined by naked eye examination of the viscera and tissues, but complete histological studies revealed visceral lesions. Acute inflammatory changes of the respiratory tract were observed in most of these cases.

Werne and Garrow stated that as there are no certain postmortem criteria of asphyxia, an opinion that death has resulted from "accidental mechanical suffocation" should be given only after detailed naked eye and histological examinations of the tissues have excluded any other cause of death. This view is supported by Bowden [18] (1950), who warned against the practice of diagnosing "accidental suffocation" on circumstantial evidence alone. When deaths are ascribed to "accidental mechanical suffocation," inquests are held, and as the magistrates usually regard such deaths as being preventable, it is evident that many mothers unnecessarily experience a sense of culpable carelessness.

The facts set out in this paper emphasize the need for a reorientation of the approach to deaths of medicolegal importance in accordance with current views in applied physiology and pathology.

*The concept of asphyxia as an entity is not acceptable to most physiologists and pathologists, and it is suggested that the term should no longer be used in the practice of forensic medicine, as it has led to so much confusion and inaccuracy in the past.*

SUMMARY

The causes of rapid anoxic death have been classified. This classification is based on the concept that all rapid anoxic deaths are initiated either by defective oxygenation of the blood in the lungs, or by a reduction of the oxygen-carrying capacity of the blood, or by a depression of the oxidative processes in the tissues.

The mechanism of failure in rapid anoxic death is described in relation to the experimental studies of Swann and Brucer. The pathology of rapid anoxic deaths is dealt with and the relationship of these deaths to deaths from secondary shock and deaths from natural causes is emphasized.

Detailed consideration is given to the significance of findings such as visceral congestion, petechial hemorrhages, cyanosis, postmortem fluidity of the blood, cardiac dilatation and hyperglycemia, in order to show that asphyxia is not a pathological entity. Attention is drawn to the errors which arise when such findings are accepted as pathognomonic of asphyxia.

It is emphasized that in the analysis of postmortem findings for medicolegal purposes, a clear distinction should always be made between nonspecific general pathological changes and special pathological changes which are dependent on the type of death.

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18. Bowden, K.: Sudden Death or Alleged Accidental Suffocation in Babies, M. J. Australia **1**:65, 1950.

# EMBRYOGENESIS OF TRACHEOESOPHAGEAL FISTULA AND ESOPHAGEAL ATRESIA

## A Hypothesis Based on Associated Vascular Anomalies

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AND
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SAN FRANCISCO

CONTRADICTORY evidence has arisen regarding the origin and the pathogenesis of tracheoesophageal fistulas and atresia. Many theories have been advanced, few of which have a sound basis in painstaking postmortem study.

Congenital atresia of the esophagus is now recognized as a frequent anomaly, at least as frequent as congenital heart disease,[1] with incidence well above one in eight hundred births. The symptoms, the management and the treatment of this condition have been exhaustively dealt with and corrective operative procedures devised. The etiology, perhaps because of its mainly academic nature, has scarcely been mentioned in recent publications on the subject.

It is the purpose of this study to present the postmortem findings on three infants, two with esophageal atresia and tracheoesophageal fistula, and one with tracheoesophageal fistula alone. In all three these anomalies were associated with anomalies of the arterial system which we feel may have a direct etiological bearing on the development of the tracheal and esophageal malformations. In the first two infants there was an aberrant subclavian artery crossing between the upper atretic esophageal cul-de-sac and the lower fistulous opening into the trachea (Fig. 1). In the third there was an aberrant anastomotic artery arising from the aorta and crossing behind the esophagus at the level of the fistula (Fig. 2). The pertinent literature and theories will be cited, reviewed, and criticized briefly. No exhaustive review will be attempted, since Plass[2] and Rosenthal[3] have done this comprehensively.

Since the surgical correction of these defects is feasible, we feel that the matter of recognizing and ligating the aberrant vessel at the time of surgical intervention becomes of importance if the anastomosis is to function successfully later in life. This is borne out in the dysphagia lusoria (Fig. 6) which occurs at a later age

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Mr. Fluss is in this country as a Fulbright grantee.

From the Division of Pathology, University of California School of Medicine.

1. Belsey, R. H. R., and Donnison, C. P.: Congenital Atresia of the Oesophagus, Brit. M. J **2**:324, 1950.

2. Plass, E. D.: Congenital Atresia of the Esophagus with Tracheo-Esophageal Fistula, Johns Hopkins Hosp. Rep. **18**:259, 1919.

3. Rosenthal, A. H.: Congenital Atresia of the Esophagus with Tracheo-Esophageal Fistula, Arch. Path. **12**:756 (Nov.) 1931.

because of arteriosclerotic changes in the aberrant vessel. We hope that this paper will stimulate a careful search for the associated vascular anomalies on the part of radiologists, surgeons and pathologists.

## REPORT OF CASES

CASE 1.—S. R., a white boy 4 days old, was born following a full-term, normal pregnancy. There was no maternal history of rubella. The first clue to the abnormality was persistent regurgitation of all feedings. A roentgenogram revealed complete obstruction of the esophagus; gas was present in the stomach and bowel. Surgical repair of the atresia and tracheoesophageal fistula was accomplished on the third day of life. At operation, the two esophageal segments were found in close apposition. The lower segment entered the trachea immediately above the bifurcation, was narrowed and tapered toward its upper extent. No other abnormality was

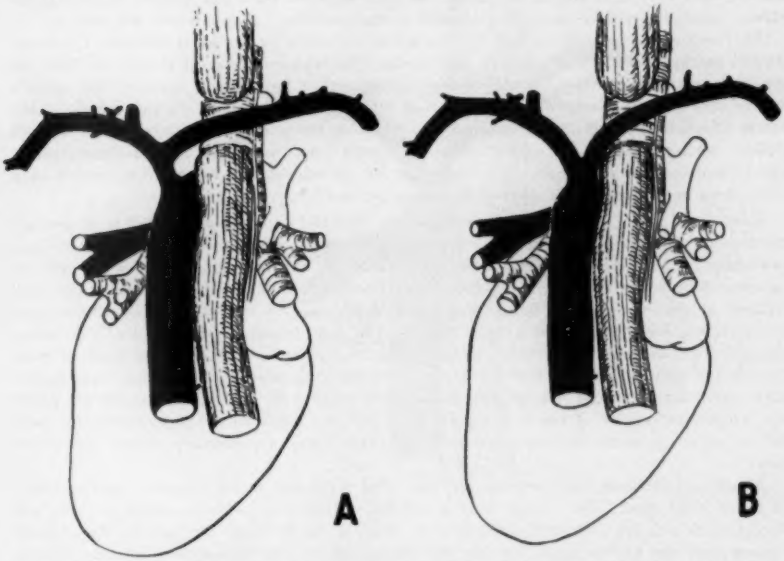


Fig. 1.—*A*, posterior view of the anomaly in Case 1. The aberrant right subclavian artery arising from the left subclavian artery passes between the two esophageal segments and behind the trachea.

*B*, posterior view of the anomaly in Case 2. The aberrant right subclavian artery arising from the aortic arch passes between the two esophageal segments and behind the trachea.

noted. A gastrostomy was performed, and the infant was returned to the ward in good condition. During the night pulmonary edema developed, and the infant died.

At autopsy there were no external abnormalities other than edema of the eyelids, cyanosis, and distention of the abdomen, from which a gastrostomy tube protruded, from a subcostal incision; a Penrose drain was in the thoracotomy wound. The peritoneal cavity contained no fluid or adhesions; the viscera were in normal relationship. The margins of the gastrostomy wound were sutured to the abdominal wall. Both pleural cavities were dry, without adhesions. The thymus weighed 8 gm.; the thyroid, 2 gm. The trachea was patent. The right innominate artery was absent; both common carotid arteries arose directly from the aortic arch. The left and right subclavian arteries arose by a common trunk from the left side of the descending aorta (Fig. 1*A*), 1.2 cm. below the origin of the left common carotid. The right subclavian artery then ascended obliquely between the vertebral column and the esophagus in close

proximity to the sutured ends of the latter and at the level of the fistulous opening into the trachea posteriorly, 0.4 cm. above the bifurcation. It proceeded along the posterior thoracic wall and over the dome of the pleura, passing in front of the scalenus anterior muscle. Its subsequent course was normal. There was no abnormality of the venous system. The heart weighed 17 gm. (normal). The contours and the chambers were of normal size. Epicardium, myocardium, and endocardium were normal. The foramen ovale was closed; the ductus arteriosus was obliterated. The valves were normal, as were the origin and distribution of the coronary arteries. The lungs weighed 75 gm. (normal, 40). They appeared edematous and congested; the cut surface exuded clear, frothy, watery fluid. There were a few subpleural ecchymoses. The liver, the gallbladder and the extra-hepatic ducts were normal. The spleen and the pancreas were normal. The gastrointestinal tract was examined from stomach to anus, and no abnormalities were found. The adrenals, the kidneys and the bladder and the genitalia were normal. The brain weighed 472 gm. (normal, 335). The vessels and the meninges were normal. The gyri were slightly widened; the sulci appeared narrowed because of generalized edema. Multiple serial coronal slices showed no abnormalities. The pituitary was normal.

Microscopic examination showed no changes in the heart or the great vessels. The lungs showed patchy atelectasis, congestion, and edema. The tracheoesophageal fistula was lined by stratified squamous epithelium, which became transitional at the tracheal junction. The smooth muscle fibers of the fistula wall were oriented in a haphazard fashion without regard to the lumen. Sections through the esophageal pouch above the anastomosis showed only smooth muscle; some thickening and edema of the wall were noted, with mild acute inflammation in and around the operative sites. The remainder of the microscopic examination showed only mild edema and congestion of the viscera and of the central nervous system.

Case 2.—V. V. G., a white girl 2 days old, was admitted 14 hours after a full-term, normal, spontaneous delivery. There was no history of maternal rubella. At birth the child was externally normal, but excessive mucus was present in the pharynx. There were cyanotic episodes, relieved by suction. Roentgenograms revealed atresia of the proximal esophageal segment at the level of the fourth thoracic vertebra, gas in the stomach and bowel, and hemivertebrae for the fifth and sixth vertebrae. The child became cyanotic immediately when removed from oxygen. There were scattered rhonchi over both lung fields and medium moist rales in the right upper lung field. The results of the remainder of the physical examination were within normal limits. The patient was taken to surgery the same afternoon, but the defect was irreparable because of the wide separation of the two segments. Postoperatively the child did not regain spontaneous respirations and died with signs of pulmonary edema three hours later.

At autopsy the body was externally normal. The peritoneal cavity contained approximately 15 cc. of clear fluid. The organs were in normal relationship, without adhesions. The left pleural cavity was dry; the right contained 8 to 10 cc. of bloody fluid. Through the thoracotomy incision protruded a drain placed into the retropleural space. The mediastinum was very slightly shifted to the left. The trachea was patent. There was an anomalous origin of the right subclavian artery from the arch of the aorta adjacent to the origin of the left subclavian artery (Fig. 1*B*). The right and left common carotid arteries arose close together from the aortic arch. The right subclavian artery after originating on the left side passed around behind the trachea, crossing between the two segments of the esophagus and then followed its usual course. There was atresia of the esophagus. The distal segment (fistula) originated from the lower portion of the trachea just at the bifurcation, was 2.1 cm. from the proximal segment, and had been surgically divided. The aorta arose normally. The pulmonary artery merged into a large, dilated ductus arteriosus. At the point where it joined the aorta there was a narrowing (coarctation of the infantile type) just distal to the origin of the left subclavian artery. This coarctation measured 4 mm. in its greatest diameter. The course of the aorta was subsequently normal. The heart weighed 22 gm. (normal, 17 gm.). Both chambers were dilated; the walls were moderately hypertrophied. There were no septal or valvular defects. The right and left coronary arteries arose normally and followed the usual pattern. The lungs weighed 85 gm. combined (normal, 40 gm.). They appeared congested but not atelectatic. The pleura of the right lung showed fibrinous reaction. The liver and biliary tract, the spleen and the pancreas were normal. The remainder of the examination was noncontributory.

Microscopic examination showed mild hypertrophy of the myocardial fibers without other alterations in the heart or the vascular system. The lungs were severely congested, as was the spleen. The esophageal mucosa showed acute ulceration and inflammation in the vicinity of the surgical exploration. The fistulous tract was lined by stratified squamous epithelium identical with that in the remainder of the esophagus. The transition to respiratory epithelium was gradual. The muscular layers (composed only of smooth muscle) were not continuous between the two structures, becoming a haphazard tangle at the site of the fistula. The remainder of the gastrointestinal tract showed no significant changes. The liver and biliary system and the pancreas were normal. The kidneys were moderately congested; the bladder and the genital system were normal. The thyroid and parathyroids and the adrenals showed no changes. In both occipital lobes of the brain were focal areas of microgyria. There were no other changes.

Fig. 2.—Posterior view of the anomaly in Case 3. Arteria aberrans passing behind the esophagus at the level of the tracheoesophageal fistula (see also Fig. 5).

CASE 3.—G. R., a white girl 8 months old, was born following a full-term, normal pregnancy. There was no maternal history of rubella. Respiration had been poor since birth, and the baby choked and became cyanotic when attempting to nurse on a bottle. Increasing abdominal distention was noticed. On admission the infant was underweight (6.7 kg.) but well developed in all other respects. There was gross abdominal distention, but no tenderness was present. Fluoroscopy demonstrated a tracheoesophageal fistula after a lipoidal swallow. The child was taken to surgery, and excision of the fistulous tract was accomplished. Following operation, respiratory difficulty developed, necessitating tracheotomy. In spite of this the infant became worse and died three days later. At autopsy there were no external abnormalities other than moderate emaciation. The thoracotomy and tracheotomy incisions were noted. The abdomen

was much distended with air. The peritoneal cavity was dry; there was marked gaseous distention of the bowel. The pleural cavities were dry; on the right there were numerous fibrinous adhesions. The pericardial sac contained the usual moisture. An extensive tracheobronchitis with occlusive mucopurulent secretions explained the respiratory difficulties necessitating tracheotomy and resulting in death. The fistula had been surgically interrupted; its orifice was at the level of the carina. The lungs were heavy and edematous, with areas of atelectasis. The heart and large parietal vessels were normal. An anomalous unpaired ostium was found in the posterior aortic wall just proximal to the first pair of intercostal arteries. A small vessel could be traced from this opening across the vertebral column (Fig. 2) ascending obliquely up directly opposite the fistulous tract in the trachea, branching into the second intercostal space on the right. At this point it was inadvertently torn from the surrounding areolar tissue and, unfortunately, further dissection was impossible. The remainder of the examination revealed no abnormalities.

In the microscopic examination changes were limited to the respiratory tract and the esophagus only. The trachea and the major bronchi were the site of acute inflammation, with ulceration and sloughing of the epithelium, the inflammation of the wall extending into the mediastinal tissues. The source was apparently the tracheotomy stoma, where the changes were most pronounced and clumps of bacteria were found. Changes in the esophageal wall were of a mild inflammatory nature. The esophageal wall at the site of the fistula showed a plexiform tangle of muscle fibers (all smooth, nonstriated elements) which were not oriented to either the esophagus or the trachea. The mucosa was of the stratified squamous type.

At this point a brief review of the embryology of the foregut and the vascular system is pertinent.

### ARTERIAL DEVELOPMENT [4]

The primary embryonic arteries are the right and left primitive aortas, joined by pairs of aortic arches which develop and disappear at various stages. By the 13-mm. stage (six weeks) the adult pattern is beginning to emerge (Fig. 3). The first and second arches disappear early; the carotid vessels are formed by the third arches. Both fifth aortic arches (which rarely develop) and the sixth arch on the right disappear. The fourth arch on the right persists as the subclavian artery, and that on the left as the definitive aorta. The sixth arch on the left persists until birth as the ductus arteriosus. The last to disappear just before the 18-mm. stage (seven-week embryo) is the caudal portion of the right fourth arch connecting the right subclavian artery to the descending aorta. During this period there are three possibilities of maldevelopment resulting from the persistence of channels that normally disappear or from the disappearance of channels that normally persist. Only those anomalies which may influence esophageal development by their proximity will be mentioned.

1. Formation of an aberrant subclavian artery (Fig. 4*A*). The estimates of the frequency of this condition vary between 4:1,000 to 16:1,000.[5] When the cranial portion of the right fourth aortic arch becomes obliterated, the caudal segment ("dorsal aortic root") persists, thus forming the right subclavian artery.[6]

4. Congdon, E. D.: Transformation of the Aortic Arch System During the Development of the Human Embryo: Contributions to Embryology, Washington, Carnegie Institution of Washington **14**:47, 1922.

5. Goldbloom, A. A.: Anomalous Right Subclavian Artery, Surg., Gynec. & Obst. **34**:378, 1922.

6. Wood, J.: Tr. Path. Soc. London **10**:119, 1859.

Fig. 3.—Normal vascular development. Arches and segments which disappear before birth are defined by interrupted lines. The last to disappear at or just before the 18-mm. stage is the caudal segment of the fourth right aortic arch.

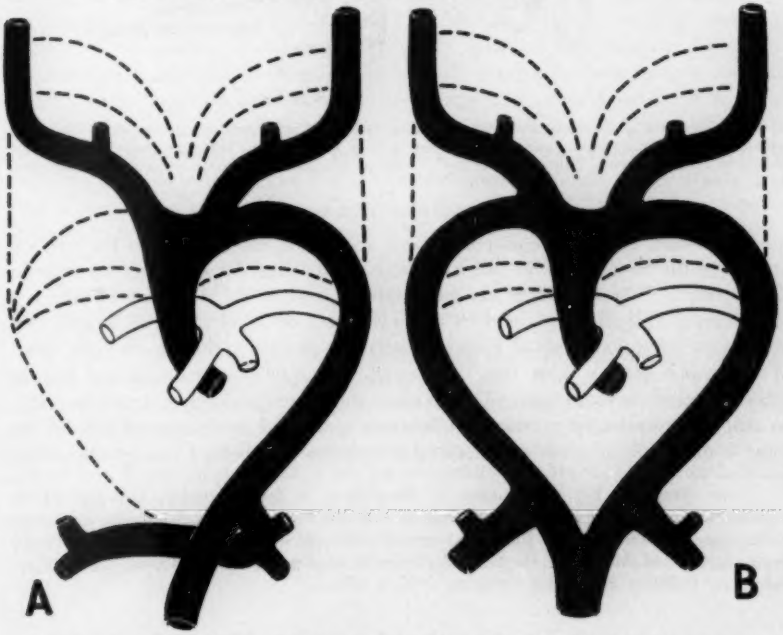


Fig. 4.—*A*, aberrant right subclavian artery arising with the left subclavian artery by a common root from the aorta. *B*, double aortic arch.

2. Persistence of a right-sided aortic arch. Variations of this anomaly are:

(*a*) Right aortic arch associated with a right descending aorta crossing over to the left side in the lower thoracic region behind the esophagus.

(*b*) Right aortic arch associated with a left descending aorta crossing behind the esophagus at a higher level.

(*c*) Double aortic arch (Fig. 4*B*). This is established when the caudal remnant of the right dorsal aorta (fourth aortic arch) persists.

(*d*) Arteria aberrans (Fig. 5). This also results from persistence of the caudal remnant of the dorsal aorta, the so-called "aberrant" artery, which ascends obliquely behind the esophagus, terminating somewhere along the vertebral column or anastomosing with another "aberrant" artery (an occasional branch of the right subclavian artery or of the costocervical trunk).

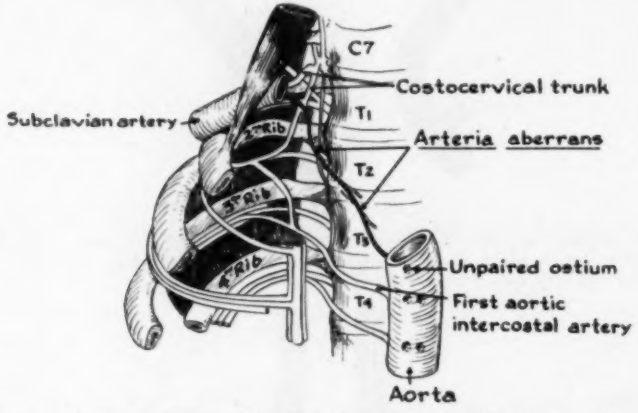


Fig. 5.—Arteria aberrans illustrated arising from an unpaired ostium, passing obliquely across the vertebral column and anastomosing with a branch from the right subclavian artery. Modified from Morris' "Human Anatomy" (Edition 10, the Blakiston Company, Philadelphia, 1942, p. 641).

## ESOPHAGEAL DEVELOPMENT [7]

In a 4-mm. embryo the esophagus is a short tube extending from the tracheal groove to the stomach. The larynx, trachea and lungs develop from an anterior diverticulum. The esophagus elongates rapidly, and by the 35th day (5 mm.) it has attained 25%, and by the 49th day (18 mm.) 40%, of the body length. At the 4-mm. stage longitudinal ridges (externally grooves) develop on both sides. With further growth these fuse, separating the posterior portion of the trachea from the primitive esophagus. The fusion of the ridges gradually extends cranially, so that the respiratory primordium becomes more and more separated from the foregut until only a small communication remains just behind the hypobranchial

7. (*a*) Arey, L. B.: Development of Esophagus, in Developmental Anatomy, ed. 5, Philadelphia, W. B. Saunders Company, 1946, p. 218. (*b*) Patten, B. M.: Esophagus, in Human Embryology, Philadelphia, The Blakiston Company, 1946, pp. 461 and 472. (*c*) Hamilton, W. J.; Boyd, J. D., and Mossman, H. W.: Development of Esophagus, in Human Embryology, Baltimore, Williams & Wilkins Company, 1945, p. 170.

eminence (the primitive laryngeal additus). If the fusion is incomplete, a fistula may result. The esophageal lumen becomes obliterated before the seventh week for a period of time. During the seventh to eighth week it undergoes recanalization, reestablishing its lumen.

## THEORIES OF CAUSATION

The various theories as regards the causation of this condition have been presented in detail by Rosenthal.[2] Inflammation and intrauterine trauma were considered factors by some but were rejected by others since histological examination did not support this. Klebs and Kraus (cited by Rosenthal) felt that excess of the original formative material is used up in the development of the trachea, bronchi and lungs, resulting in esophageal atresia. This theory does not explain fistula formation.

The remaining most widely accepted theories Rosenthal divided into three groups:

1. Theories dealing with retardation of esophageal development before recanalization of its lumen: Kreuter [8] stated that interference with recanalization results in permanent atresia, but he neither suggested any causes nor explained the concomitant fistula formation. His work was contradicted by Keibel and Mall,[9] who maintained that the esophageal lumen never is permanently obliterated. Most embryologists now agree that recanalization occurs.[7] As recanalization occurs at the 18-mm. stage only, finding this anomaly in embryos smaller than the 18-mm. stage would be a serious objection to this theory. Two instances have been reported in the literature. In one the anomaly is described as occurring in a 9-mm. embryo with esophageal stenosis and tracheoesophageal fistula.[10] The description is not convincing, since in the diagram the upper "blind" pouch of the esophagus appears as a mere shallow diverticulum. At this stage no complete separation of trachea and esophagus exists; therefore the "fistula" is always physiological. Ysander [11] described an 8-mm. embryo, a thoracopagus monster, in which this condition was bilateral. However, as his case was that of a thoracopagus a measurement of 8 mm. does not necessarily give the true age of the embryo. Furthermore, as one vascular system was completely developed, the embryo might have been older than the measurement implied. Also, his description of the arterial development is brief and incomplete, precluding accurate evaluation as to the presence or the absence of vascular anomalies.

2. Theories concerning deviation of the tracheoesophageal septum:

Shattock's [12] widely accepted explanation can be taken as typical of the following septal derivation theories. "When the lower air passages develop from the

8. Kreuter, E.: Die angeborenen Verschliessungen und Verengerungen des Darmkanals in Lichte der Entwicklungsgeschichte, Deutsche Ztschr. Chir. **70**:1, 1905.

9. Keibel, F., and Mall, F. P.: Separation of Esophagus from the Trachea: Human Embryology, Philadelphia, J. B. Lippincott Company, 1912, Vol. 2, pp. 312 and 313.

10. Gruenwald, P.: A Case of Atresia of Esophagus Combined with Tracheo-Esophageal Fistula in a 9 Mm. Human Embryo and Its Embryological Explanation, Anat. Rec. **78**:293, 1940.

11. Ysander, F.: Studies on the Morphology and Morphogenesis of Human Thoracopagic Monsters, Inaugural Dissertation, Upsala, 1924.

12. Shattock, S. G.: Congenital Atresia of the Esophagus, Tr. Path. Soc. London **41**:87, 1890.

anterior wall of the mesenteron, the posterior wall is PULLED FORWARD and, when the larynx develops later, it uses up the narrow lumen and the lower segment of the esophagus is left connecting with the air passages" (capitals ours).

Forssner, cited by Keibel and Mall,[9] believed that the lower portion of the tracheoesophageal septum fails to develop, thus leaving the esophagus in communication with the lower part of the trachea. He called attention to a lateral furrow on the wall situated externally on each side of the esophagus caudad to the ventral pulmonary anlage and presenting as a corresponding internal elevation. This is so situated that if the walls of the esophagus should coalesce along this groove a ventral portion would be cut off communicating freely with the trachea near its bifurcation. The presence of these grooves and the accompanying theory, attractive as it may seem, are not confirmed by any other author. Unless there is substantial evidence to support it, it remains unproved.

Huntington,[13] elaborating the above theory, suggested that in some instances the lateral body processes to which the esophageal grooves correspond might become edematous and compress the esophageal walls to cause stenosis. This might conceivably explain the fistula formation, but it is difficult to visualize a vertical longitudinal "process" as being responsible for stenosis affecting only the dorsal portion of the esophagus and leaving uninvolved the ventral portion, even granting that the dorsal esophagus has a narrow lumen as he states. Even greater difficulty is encountered when one attempts to explain all the variations encountered in this anomaly by Forssner's theory.

3. Directly associated with the above are the theories concerning changes of tension as the causative factor in any deviation of the septum:

Zausch[14] expressed the belief that the direction of pressure exerted by the heart anlage determines the type of anomaly produced. Thus, if it pushes in a cephalad direction, it prevents proper closure of the palatal clefts. If it is directed toward the pharynx, the author would have us believe the pressure, acting on the "narrow isthmus" of the esophageal anlage, results in the anomaly. Schmitz[15] and Ysander[11] independently have agreed with this theory that a large heart may cause a pressure which is exerted mainly at the dorsal part of the esophagus, owing to the curved position of the embryo, with the breakdown of the posterior esophageal wall and resulting atresia. The pressure of the heart on the lower end of the esophagus leads to fusion of its posterior wall with the longitudinal lateral crests and results in a fistula.

Keith and Spicer[16] and Kraus[17] were the only authors to lay stress on the presence of anomalous great vessels in the thorax. The former described three

13. Huntington, J.: Report of a Case of Congenital Atresia of Esophagus, Boston M. & S. J. **180**:354, 1919.

14. Zausch, P.: Ein Fall von Oesophagus Atresie und Oesophago-Trachealfistel, Arch. path. Anat. **234**:94, 1921.

15. Schmitz, J. A.: Ueber die formale Genese der Oesophagus-missbildungen, Arch. path. Anat. **247**:278, 1923.

16. Keith, A., and Spicer, J. E.: Three Cases of Malformation of the Tracheo-Esophageal Septum, J. Anat. & Physiol. **41**:52, 1906.

17. Kraus, I.: Die Erkrankungen der Mundhöhle und der Speiseröhre, in Nothnagel's Handbuch der speziellen Pathologie und Therapie, Vienna, 1902, Vol. 41, p. 96.

cases of a right aortic arch with the left subclavian artery passing behind the trachea just below the atretic esophageal cul-de-sac. Kraus suggested that persistence of a muscular cord uniting the two sections of the esophagus is due to fetal atrophy from pressure of anomalous arteries. He reported four successive cases of esophageal obstruction, in three of which there was an abnormal origin of the right subclavian artery. Neuhauser [18] recently found this anomaly in two of eight cases of esophagotracheal fistula without atresia by radiological methods.

Criticisms directed against the purely mechanical explanation of the abnormality —a pressure of an abnormal structure such as the cardiac anlage and a subsequent septal deviation—are that a large cardiac anlage acting abnormally would rarely develop into a normal heart; secondly, the trachea would tend to be involved more directly, since it is closer to the cardiac anlage than the esophagus. Lastly, the reconstructed models of human embryos from 3 to 5 mm. in length show that the cardiac anlage is always large but separated by an appreciable distance from the primary foregut.

Most authors concur that the pressure from an aberrant vessel is still less likely to cause this abnormality. According to Plass,[2] vascular anomalies occur too rarely in this connection; he feels it is more probably a concomitant anomaly rather than a causative factor. Another reason why vascular malformations did not receive the attention deserved is that there has been a mistaken supposition that the anomaly has to develop prior to the 4-mm. stage, when the separation of trachea and esophagus is as yet incomplete. The apparent sources of such misconceptions are the statements by Keibel and Mall [9] "the 4 mm. embryo . . . is close to the stage in which the anomaly must arise" and "in the 4.9 mm. embryo the separation of the esophagus and trachea has proceeded so far that the anomaly could hardly develop." These statements have been cited many times as evidence against theories promulgating anomalous vessels as causing the tracheoesophageal malformations. Textbooks of embryology are vague in pinpointing the stage of development at which the separation is completed; reconstructed embryos clearly show that the "separation" between esophagus and trachea is rarely evident in the 4 mm. stage and still incomplete even in the 11-mm. stage.

A reason for this view of esophageal and respiratory-tract development might be the mistaken assumption that the lung bud represents the entire respiratory primordium,[19c] ignoring the portion of the foregut which potentially forms the larynx and the trachea proximally. That the larynx and the trachea may have an origin independent of that of the bronchial tree is suggested by the demonstration of the exceedingly rare anomalous absence of the trachea [19] in which the larynx was well developed and terminated in a blind pouch while the bronchi and the lungs were normally formed.

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18. Neuhauser, E. B. D.: Personal communication to the authors.

19. (*a*) Marek, Y. Y.: Congenital Deformity of the Trachea, Ohio M. J. **36**:1308, 1940. (*b*) Payne, W. A.: Congenital Absence of the Trachea, Brooklyn M. J. **14**:568, 1900. (*c*) Milles, G., and Dorsey, D. B.: Intra-Uterine Respiration-like Movements in Relation to Development of the Fetal Vascular System: A Discussion of Intra-Uterine Physiology Based upon Cases of Congenital Absence of the Trachea, Abnormal Vascular Development, and Other Anomalies, Am. J. Path. **26**:411, 1950.

Secondly, since the aortic arches come to lie in close proximity to the esophagus at the 18-mm. stage, it follows that the septum must have grown craniad to extend the esophagus and delimit the trachea and larynx from the primitive foregut (compare the caudal migration of the aortic arches secondary to the cephalad growth of the neural tube and pharynx).

Thirdly, if the anomaly occurred at the 4-mm. stage as proposed by various authors, the fistula would be at a much higher level than observed in actual pathological material, since at this stage the communication between the respiratory primordium and the foregut in the vicinity of the future larynx is physiological.

Microscopic findings are also inconsistent with the belief that the anomaly develops at the 4-mm. stage. The esophageal and tracheal smooth muscle fibers are formed from mesenchymal condensations around their respective tubes. Our sections show a great disturbance of the arrangement of smooth muscular fibers in the region of the defect, a finding one would expect were the interference after rather than before the formation of the muscle layers.

Another feature which has been overlooked by the authors who attempt to pinpoint the development of the esophagotracheal fistula and atresia to an exact moment of embryological development (4-mm. stage) is the wide variations in the abnormalities encountered. These are:

1. Esophageal atresia only (no fistula)
2. Esophageal atresia with
   (*a*) Upper segment fistula
   (*b*) Lower segment fistula { intratracheal / intrabronchial }
   (*c*) Double fistula (upper and lower)
3. Tracheoesophageal fistula without atresia (rare)

Such a wide variation in the abnormalities encountered, as well as variations in the distance between the two esophageal segments, would hardly take place should all of them occur at the same time. It is permissible to postulate that the type of abnormality varies with the relationship existing between the arterial system and the esophagus, which changes considerably during esophageal growth. If the esophageal epithelial occlusion and recanalization occur at an earlier stage than 18 mm. (13 to 18), the two esophageal segments will be more widely separated and the fistula will be high in the trachea because of the continual relative descent of the arterial tree on the developing foregut. If, however, the recanalization occurs later, the segments will be more closely approximated and the fistula lower in the tracheobronchial tree.

Rosenthal concluded that there is insufficient factual evidence to confirm any of the mechanical theories and that the anomaly rests possibly on a genetic basis such as altered or deficient cell growth.

## COMMENT

On the evidence of only three cases it would be presumptuous to make any definite conclusions as to the causes of this condition. However, the consecutive cases of vascular anomalies associated with esophageal obstruction found in individual authors' series (Keith and Spicer, Kraus, ourselves), as well as Neuhauser's

findings, suggest that careful search at autopsies or operations in cases of esophageal atresia would bring to light many more such relationships than have heretofore been reported. In one of our cases (S. R.) the anomaly was not even noticed at operation. In the other case (V. V. B.) the exact nature of the anomalous vessel was not appreciated. In Case 3 the anomaly was so minute that recognition in the course of surgical intervention was obviously impossible. Before we can conclusively establish the suspected etiological relationship of esophageal atresia (with or without tracheoesophageal fistula) and anomalous arterial channels it will be necessary to demonstrate these anomalies by radiological, surgical or necropsy methods. The utmost care and diligence must be used in autopsy dissections if one of these vascular anomalies is to be disclosed. We found that the best way to trace even minute aberrant vessels is to open the thoracic aorta along its anterior border *in situ* and trace all vessels with unpaired openings in the posterior wall between the left subclavian artery and the first paired intercostal branches. The unpaired bronchial vessels which usually arise from the anterior wall of the aorta can be excluded.

Preoperative diagnosis of these vascular anomalies is now possible, thanks to the radiological techniques established by Neuhauser.[20]

All the above theories are in part correct; theoretically, an abnormal artery fits into the picture as the last missing piece of the puzzle. The anomaly occurs between the 13-mm. and the 22-mm. stage, before the esophageal lumen has recanalized and before the esophagus and the trachea have fully separated. At this stage the arterial system is already developed, in close association with the esophagus (as can be seen in the excellent drawings and descriptions of Hamilton, Boyd, and Mossman [7c]). Any one of the vascular anomalies discussed may interfere with esophageal recanalization either by direct compression or by causing a deviation of the septum, both resulting later in fistula formation by pressure atrophy. Thus, if in Shattock's theory we substitute "pushed forward" (instead of "pulled forward") by an anomalous artery, for example, the same result will occur. We believe that pressure directed by an anomalous artery lying in close proximity to the developing esophagus, rather than a cardiac anlage, which is anterior to the trachea, more plausibly explains the anomaly.

If even a minute search fails to reveal an abnormal artery, it is possible that a mere delay in the differentiation of the arterial system, that is, a temporary persistence (beyond the eighth week of gestation) of the connection between the fourth right arch and the descending aorta behind the esophagus would account for these cases of esophageal stricture (Fig. 3).

Another objection will be raised in instances where an anomalous vessel crosses behind the esophagus unassociated with esophageal stenosis and fistula formation. That such cases occur is evident from the few reported instances of the so-called dysphagia lusoria (Fig. 6). This objection can be answered by postulating that recanalization of the esophagus occurred at an early stage (i. e., 13 mm.), when the vascular arches are too far craniad to interfere materially with esophageal development.

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20. Neuhauser, E. B. D.: The Roentgen Diagnosis of Double Aortic Arch and Other Anomalies of the Great Vessels, Am. J. Roentgenol. **56**:1, 1946.

Fetal blood vessels also vary in size according to the average intravascular pressure and volume of blood flow,[21] as evidenced by the alterations in relative vessel size in certain congenital lesions of the heart. Therefore, the size of the aberrant vessel found at birth does not necessarily correspond to the size of the same vessel at the 13- to 18-mm. stage. Thus an aberrant vessel large enough to cause atresia in early fetal life may have decreased in size so that only a small vessel is present at birth (as in our third case). On the other hand, a channel which at the 18-mm. stage was barely open may have kept on enlarging throughout fetal growth to a full-size aberrant vessel, owing to altered hemodynamics. Some evidence for this has been offered by Milles and Dorsey,[19c] and it is likely that vascular anomalies can be explained on the theory of demand and supply.

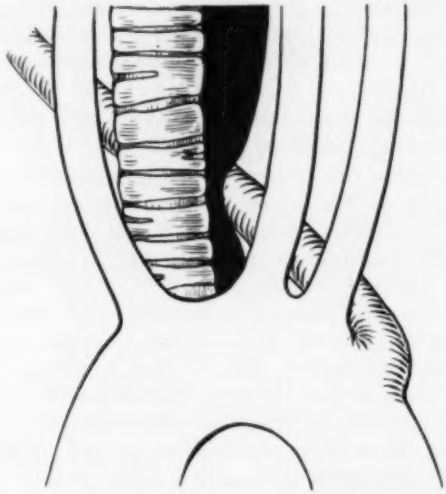

Fig. 6.—An abnormal right subclavian artery causing dysphagia (see text, page 16).

Other associated anomalies are commonly atresia ani and a thoracic hemivertebra, as in our second case. Plass [2] found atresia ani in 24 of 94 cases of esophageal maldevelopment. Our first impulse would be to attribute such widely separated anomalies to a primary chromosomal defect or a common-factor deficiency in the formation of the primordial tissues. However, since perforation of the buccopharyngeal septum and recanalization of the intestinal tract precede perforation of the cloacal membrane, pressure by swallowed amniotic fluid may be necessary to facilitate the last. In esophageal atresia no amniotic fluid can be swallowed. Similarly, the finding of a hemivertebra in the thorax may be attributed to the presence of an anomalous vessel interfering with local circulation rather than to a fundamental change resting on a genetic basis.

21. Hamilton, W. F.; Woodbury, R. A., and Woods, E. B.: The Relation Between Systemic and Pulmonary Blood Pressures in the Fetus, Am. J. Physiol. **119**:206, 1937, cited by Milles and Dorsey.[19e]

SUMMARY AND CONCLUSIONS

Three cases of esophageal malformations (two with stenosis and tracheoesophageal fistula; one with fistula alone) associated with anomalies of the arterial system are presented. The arterial anomalies were, in two cases, an aberrant right subclavian artery and, in the third, an "arteria aberrans" crossing behind the trachea at the site of the defects.

The esophageal and the pertinent vascular embryology are discussed, with stress being placed on the relationship of the presence of anomalous arteries and the pathogenesis of tracheoesophageal malformations.

Various theories as to the pathogenesis of these anomalies are reviewed and criticized.

Theoretical evidence for a mechanical (vascular) hypothesis is presented and is supported by the findings in three cases.

The necessity for recognizing arterial anomalies at the time of corrective surgery is pointed out.

Dr. W. C. Deamer, San Francisco, permitted us to use his cases.

# Case Reports

## ADRENAL PHEOCHROMOCYTOMA WITH NEUROBLASTOMATOUS ELEMENTS

### Report of a Case with Autopsy

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THE CELLS constituting the medulla of the adult adrenal gland have a common ancestor, the sympathogonia. Cells of this type invade the anlage of the adrenal cortex during the eighth week of intrauterine life and subsequently aggregate in the center (Wiesel [1]). These differentiate into ganglion cells and chromaffin cells. These cells arrested in any stage of development may give rise to medullary neoplasms, producing a tumor composed of the arrested cell type or of a mixture with different degrees of differentiation. Such tumors of mixed cell types have been observed both in the adrenal medulla and in other situations containing sympathetic nervous tissue. These have been referred to by Herxheimer,[2] Wahl,[3] Dunn,[4] Cushing and Burt,[5] Blacklock,[6] and Wahl and Craig.[7] Most of these, however, refer to tumors showing a combination of primitive cells and adult neurons, e. g., ganglioneuroblastoma. A mixture of adult chromaffin cells (pheochromocytes) and more primitive cells in a single tumor is an infrequent finding. The few reported (Lewis and Geschickter [8]; Wahl and Robinson [9]) have been seen in situations other than the adrenal gland. It has been stated (Willis [10]) that although the primitive cells in tumors, viz, the neuroblasts, may display potentiality to differentiate into chromaffin cells as well as into nerve cells, in the great majority of cases any differentiation displayed is in the direction of formation of nerve cells only. Hence pheochromocytic elements tend to occur in the pure state, unmixed with more primitive cell types. The case to be reported is that of a tumor of the adrenal medulla showing the rare combination of neuroblasts and pheochromocytes with evidence of functional activity of the latter type of cells.

From the Departments of Medicine, and Pathology, University of Ceylon.

1. Wiesel, J.: Anat. Hefte **19**:481, 1902.
2. Herxheimer, G.: Beitr. path. Anat. u. allg. Path. **57**:112, 1913.
3. Wahl, H. R.: J. M. Research **30**:205, 1914.
4. Dunn, J. S.: J. Path. & Bact. **19**:456, 1914-1915.
5. Cushing, H., and Burt, W. S.: Am. J. Path. **3**:203, 1927.
6. Blacklock, J. W. S.: J. Path. & Bact. **39**:27, 1934
7. Wahl, H. R., and Craig, P. E.: Am. J. Path. **14**:797, 1938.
8. Lewis, D., and Geschickter, C. F.: Tumors of the Sympathetic Nervous System: Neuroblastoma, Paraganglioma, Ganglioneuroma, Arch. Surg. **28**:16 (Jan.) 1934.
9. Wahl, H. R., and Robinson, D.: Neuroblastoma of Mediastinum with Pheochromoblastomatous Elements, Arch. Path. **35**:571 (April) 1943.
10. Willis, R. A.: Pathology of Tumors, St. Louis, C. V. Mosby Company, 1948, p. 846.

## CLINICAL DATA

D. H. S., aged 25, a Sinhalese man, was admitted on June 18, 1949, to a surgical ward of the General Hospital, Colombo, Ceylon, because of severe abdominal pain and vomiting, but as no surgical condition was found, he was later transferred to a medical ward. In January 1948, he had an attack of severe abdominal pain with jaundice, which was cured after a six weeks' stay in the hospital. Two weeks later he vomited on two occasions, each attack being followed by profuse sweating. Since then he had suffered from frequent similar attacks of vomiting, which had no relationship to meals. He had vomited fair quantities of blood on four occasions and felt faint each time. Although he noticed no hemorrhoids, he had passed blood mixed with feces on several occasions. Three months before admission he suffered from polyuria and increased frequency of micturition, particularly at night. He also began to have dimness of vision during the last six weeks.

He was employed as a rubber tapper and performed a strenuous day's work prior to his jaundice. He was quite temperate in his habits, and his diet was vegetarian. There was nothing of relevance in his past or family histories.

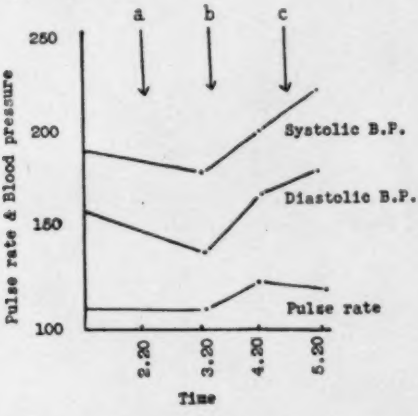


Fig. 1.—Blood pressure response to amobarbital sodium (sodium amytal®): (*a*) 2 grains (0.13 Gm.) administered; (*b*) 2 grains administered; (*c*) 3 grains (0.19 Gm.) administered.

He was thin and sallow in complexion. His skin showed old ecchymoses chiefly over the trunk. He was emotionally unstable, crying for no special reason, but answered questions intelligently; he admitted that since his illness he had been very irritable.

His heart was moderately enlarged, and the second aortic sound was accentuated. There was gallop rhythm, best heard in the mitral area. The pulse was regular, and its rate varied between 100 and 150 per minute. The blood pressure ranged between S/D 190/150 and 200/165 mm. Hg. The brachial and radial arteries were definitely thickened.

No abnormality was found in the respiratory system.

The kidneys were not palpable, and no other tumors were felt in the abdomen. His daily output of urine was about 43 oz. (1 l.), and, except for a trace of albumin and 2 to 3 red blood cells per high power field, nothing abnormal was found.

His acuity of vision was poor, and he felt as if a web were placed before his eyes. Ophthalmoscopic examination showed a fairly definite degree of papilledema with retinal hemorrhages and exudate. The retinal arteries were thickened.

*Laboratory Investigations.*—The red blood cells numbered 3,140,000 per cubic millimeter; the hemoglobin content was 60 per cent. The blood urea amounted to 41 mg. per 100 cc. Urea clearance, first hour, was 60 per cent normal; second hour, 54 per cent normal. The Wassermann and Kahn reactions were negative.

*Progress.*—The vomiting continued on and off during the next week, and in addition there were attacks characterized by sweating, dyspnea, chills, tremors and palpitation which lasted for 15 to 30 minutes. During these attacks the blood pressure rose to S/D 200/165 and the pulse rate to 150 per minute. There was no response to the cold pressor test, but the blood pressure showed a curious response to amobarbital sodium (sodium amytal®) (Fig. 1). There was an initial slight fall one hour after the oral administration of 2 grains (0.13 Gm.) of the drug. Subsequent administration caused a persistent rise in the blood pressure. The pulse rate was unchanged after the first dose but rose after the second. On July 6, 1949, the patient became restless and cyanosed and died quite unexpectedly.

## PATHOLOGIC OBSERVATIONS

*Macroscopic Examination.*—The tumor was fairly vascular on the surface, roughly spherical in shape, measuring about 3 in. (7.5 cm.), and occupied the position of the right adrenal gland. The entire mass was definitely delineated and

Fig. 2.—Cut section showing tumor tissue.

shelled out readily from its bed. The surrounding tissue was not infiltrated. The tumor was cystic and contained a large amount of clotted and fluid blood. No adrenal tissue could be identified. Tumor tissue, partly soft and necrotic, projected into the cystic cavity (fig. 2).

The other relevant necropsy findings were in the heart and the kidneys. The heart was enlarged and weighed 480 Gm. There was considerable hypertrophy of the left ventricular muscle.

The kidneys were contracted, the right and the left weighing 105 and 120 Gm., respectively. A significant finding was the sclerosis of the renal arteries as well as of the smaller branches which supplied the renal substance.

*Microscopic Appearance of the Tumor.*—The large and smaller lymphocyte-like cells of which the tumor was composed are shown in figure 3 *A*. The smaller cells resemble lymphocytes (fig. 3 *B*). Only the nuclei of these cells can be made out. In certain places these are arranged in the form of rosettes encircling a hyaline stroma. The large cells (fig. 4 *A*) are polyhedral in shape and show extraordinary

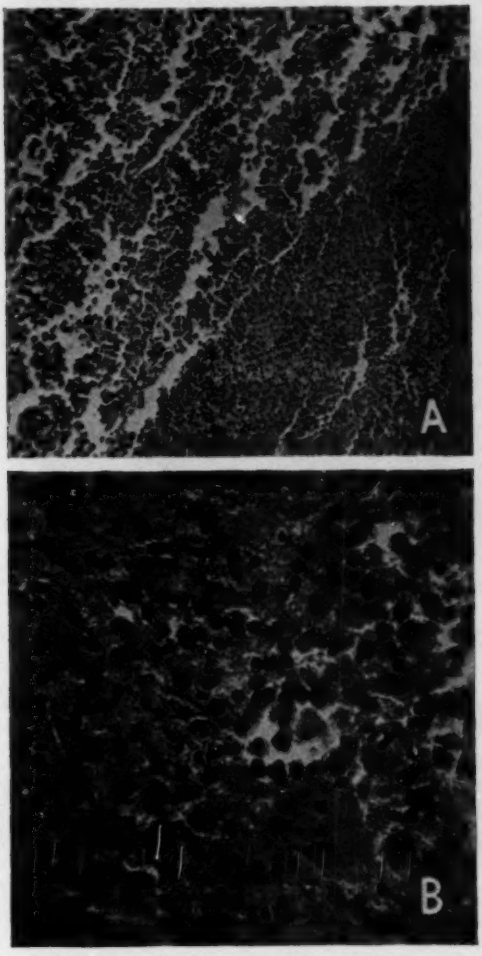


Fig. 3.—*A*, large cells (left) and the smaller lymphocyte-like cells (right) in same field; hematoxylin and eosin; × 100. *B*, high power view of the smaller cells seen in *A*; hematoxylin and eosin; × 400.

variability in size, their greatest diameters ranging from 10 to 60 microns. Their cytoplasm is acidophilic and granular, and the nuclei are hyperchromatic—several cells are very large and multinucleated (fig. 4 *A*).

In some parts of the tumor one or the other type of cell predominated, while other areas showed a fairly even admixture of both cell types. The tumor was very cellular, with a minimal amount of hyaline stroma. Small endothelium-lined vas-

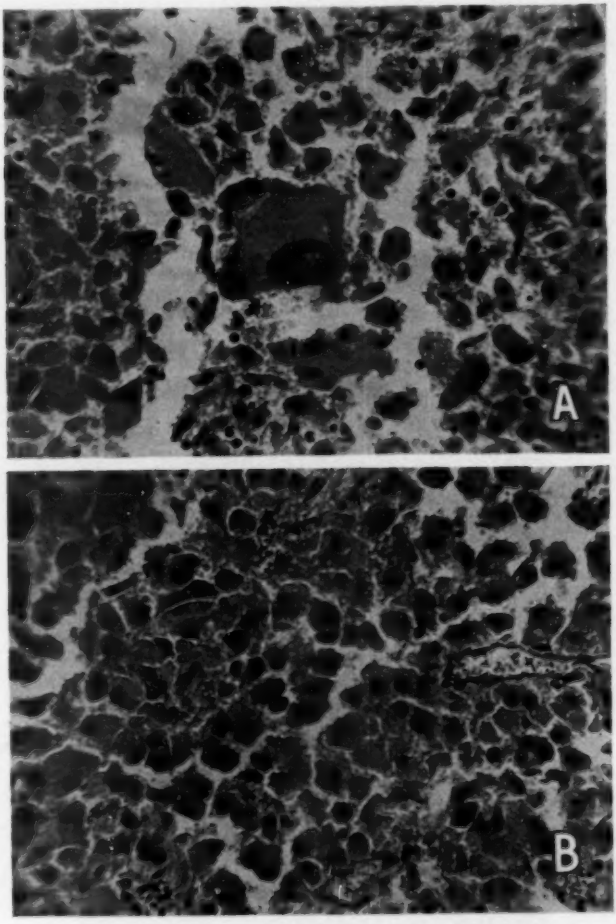


Fig. 4.—*A*, from a part of field shown in figure 3*A*, showing large multinucleated cells; hematoxylin and eosin; × 325. *B*, high power view of the large cells seen in figure 3*A*; hematoxylin and eosin; × 325.

cular spaces interrupted the tumor tissue in places (fig. 4 *B*), but definite alveoli bounded by such spaces were lacking. Areas of hemorrhage were a conspicuous feature.

## COMMENT

This case presented as a surgical emergency in that the patient was seized with severe abdominal pain and vomiting. On account of the persistent elevation of the blood pressure, the presence of red cells in the urine, the papilledema and the bleeding from the gastrointestinal tract, it was at first thought to be a case of malignant hypertension. The absence, however, of high blood urea and a fairly satisfactory urea clearance test made this diagnosis less probable. The emotional instability and the attacks characterized by sweating, dyspnea, chills, tremors and palpitation pointed to the adrenal sympathetic syndrome and it was then obvious that we were dealing with a case of an epinephrine-secreting tumor of the adrenal medulla which sometimes presents clinically as a persistent hypertension of the rapidly advancing type.

The clinical diagnosis was confirmed at autopsy by the finding of a tumor in the position of the right adrenal gland. Histological examination, however, showed that this was not a pure pheochromocytoma, but a tumor composed of two distinct cell types. Although it was not possible to carry out the chromaffin reaction and epinephrine assays, on account of prolonged fixation in formaldehyde solution, it is quite apparent that the large polyhedral cells, some with multiple nuclei, conform to the description of the type cell of the pheochromocytoma (Ewing [11]; Willis [10]; Belt and Powell [12]; Blacklock and associates [13]). This conclusion is strengthened by evidence of epinephrine formation and its effects (see above). The other type of cell in this tumor was a much smaller cell, resembling the lymphocyte. Cells of this type were arranged in the form of rosettes, the appearance resembling that of neuroblastoma of the adrenal medulla. As Wahl and Robinson [9] pointed out, the term "neuroblastoma" includes both sympathogonioma, composed of undifferentiated sympathetic cells, and sympathicoblastoma, containing more differentiated elements from which arise the adult sympathetic ganglion cells and the chromaffin cells (pheochromocytes). A tumor derived from these primitive precursors may, during the process of growth, undergo different degrees of differentiation, giving rise to tumors of mixed cell types, although, as stated earlier, the present combination of neuroblastomatous and pheochromocytomatous elements with absence of adult ganglion cells in a single tumor is rare. It is interesting to speculate whether this tumor represents a neuroblastoma differentiating in a one-side fashion into a pheochromocytoma or a pheochromocytoma differentiating structurally into a more primitive cell type. The age of onset, viz., 25 years, a time of life when neuroblastomas are rare, and the pleomorphic appearance of the tumor, with the presence of several multinucleated cells, would suggest some degree of dedifferentiation. However, a final conclusion cannot be reached, as neuroblastomas (pure and unmixed types) have been reported to occur in adults (e. g., Alyea,[14] neuroblastoma in a man of 55; Wahl and Craig,[7] neuroblastoma, ganglioneuroblastoma and ganglioneuroma in a man of 28).

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11. Ewing, J.: Neoplastic Diseases: A Treatise on Tumors, ed. 4, Philadelphia, W. B. Saunders Company, 1940, pp. 839-840.

12. Belt, A. E., and Powell, T. O.: Surg., Gynec. & Obst. **59**:9, 1934.

13. Blacklock, J. W. S.; Ferguson, J. W.; Mack, W. S.; Shafar, J., and Symington, T.: Brit. J. Surg. **35**:179, 1947.

14. Alyea, E. P.: South M. J. **26**:753, 1933.

Drugs employed in the diagnosis of pheochromocytoma fall into two groups: (1) epinephrine antagonists and (2) epinephrine provocatives. In the former category are benzodioxane and its derivatives which, as Goldenberg, Snyder and Aranow [15] have shown, cause a significant drop in blood pressure in persons with epinephrine-induced hypertension and in patients with pheochromocytoma during hypertensive states. A similar effect is produced by dibenamine hydrochloride, used by Spear and Griswold.[16] Epinephrine provocatives, on the other hand, precipitate an attack of hypertension in a resting phase. Thus, histamine, methacholine bromide (mecholyl bromide®) and tetraethylammonium bromide cause an initial drop in the blood pressure followed by a sharp rise associated with symptoms such as precordial and abdominal pain, headache, nausea and diaphoresis (Roth and Kvale [17]; Guarneri and Evans [18]; La Due, Murison and Pack [19]). Amobarbital sodium administered in the present case acted as an epinephrine-provocative drug, causing an initial slight lowering of the blood pressure, which was followed by a persistent rise. Since amobarbital sodium is commonly employed to ascertain whether hypertension is labile or not, an unusual response of this type should suggest an epinephrine-secreting tumor as the cause.

## SUMMARY

A case is reported in which a tumor of the adrenal medulla composed of pheochromocytomatous and neuroblastomatous elements presented clinically as one of malignant hypertension. The histological features are described, and emphasis is laid on the rarity of the case in which these two cell types occur in a single tumor.

The action of amobarbital sodium (sodium amytal®) was found to be similar to that of other epinephrine-provocative drugs used in the diagnosis of these tumors.

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17. Roth, G. M., and Kvale, W. F.: Am. J. M. Sc. **210**:653, 1945.

18. Guarneri, V., and Evans, J. A., cited by Cahill, G. F., and Aranow, H., Jr.: Ann. Int. Med. **31**:389, 1949.

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# Laboratory Methods and Technical Notes

## AN IMPROVED METHOD FOR HISTOLOGIC DEMONSTRATION OF ACID MUCOPOLYSACCHARIDES IN TISSUES

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SAN FRANCISCO

THE MUCINOUS intercellular substances generally classed as mucopolysaccharides constitute important elements of connective tissue. A significant handicap to adequate histologic and pathologic study has been the lack of an effective method of precise demonstration of these materials. Some such substances exhibit the property of metachromasia when stained with thionin or toluidine blue. These stains, however, have definite limitations and, in general, do not demonstrate all such substances well, particularly when the material is present in small amounts.

In connection with studies of the rheumatic diseases and arteriosclerosis it has proved important to study the detail and relationships of the several elements of connective tissues and particularly of the mucopolysaccharides. In 1946 Hale [1] briefly described a new method for staining the mucinous element of connective tissue. The method was based on the affinity of the acid mucopolysaccharides for colloidal iron, which are subsequently rendered blue by application of potassium ferrocyanide (i. e., the Prussian blue reaction). The method as presented by Hale required special fixation and presented numerous inadequacies, chief of which was the lack of precise delineation of the mucoid materials. Working from the basic principle presented by Hale, we have devised a method of staining which appears to be quite selective and has enabled a more precise demonstration of the acid mucopolysaccharides and also permits counterstaining of nuclei, collagen fibers, and "basement membranes." We have prepared an iron complex which is stable in a more acid solution than is the colloidal iron used by Hale. Carried out in the higher acid concentration the stain has proved to be more selective for the acid mucopolysaccharides. Pre-treatment of some of the sections with hyaluronidase has further elucidated the interrelationships of the reticulum, collagen, elastic tissue, and the mucopolysaccharides. It is the purpose of this paper to detail and illustrate the staining method. Subsequent reports will be concerned with the application of the method to histologic studies of various tissues, particularly of kidney, heart, blood vessels, skin, and articular tissues, both normal and pathologic.

From the Division of Pathology, University of California School of Medicine.

This investigation was aided by a research grant from the National Heart Institute, United States Public Health Service.

1. Hale, C. W.: Histochemical Demonstration of Acid Polysaccharides in Animal Tissues, Nature **157**:802, 1946.

THE METHOD

Special reagents are used: (1) a specially prepared colloidal iron and (2) ammonium aluminum-sulfate cochineal solution.

Methods for the preparation of these reagents are set forth below. Other stains and reagents are standard items available in the laboratory.

*Preparation of Colloidal Iron.*—A colloidal ammonium ferric glycerate is prepared from a concentrated solution of ferric chloride. In standardizing the solution some difficulty has been encountered, due to the variable concentration of ferric chloride in several brands of this substance. We have found ferric chloride (chemically pure, Baker) satisfactory.

The following formula is used in the preparation of the colloidal iron:

Ferric chloride (granular or lump, chemically pure, Baker) 300 gm. lump or 275 gm. granular; distilled water, 1,000 ml.; glycerin, 400 ml.; ammonia 28 per cent, 220 ml.

Dissolve the ferric chloride in water; when completely dissolved add the glycerin. Gradually add the ammonia, starting with 100 ml. A precipitate will form which will then dissolve on vigorous stirring. When this precipitate is completely dissolved, add another 50 ml. of ammonia and stir until the precipitate dissolves (about five minutes). Then add another 30 ml. of ammonia and stir for eight to 10 minutes. The reaction is rather slow when it is approaching the end point; therefore time between each addition of ammonia should be prolonged, with a gradual decrease in the amount of the ammonia added. The remaining 40 ml. of ammonia is added in two equal portions at 15 minute intervals, with vigorous stirring after each addition. The product of this reaction is a clear, deep reddish brown colloidal solution of ammonium ferric glycerate. This solution is then dialyzed by pouring into large seamless cellophane bags (sausage bags [2]) after softening the bags by immersion in water. Tie both ends of the bag and dialyze against distilled water, placing the bag in a large container filled with distilled water. Because there is increase of the volume of solution inside the bag during the process of dialysis, the bags are initially charged with about 40% of their capacity. About 1,500 cc. of the colloidal solution will increase to a volume of 4,000 cc. by the end of the dialyzing process. Adequate dialysis usually requires eight to 10 changes of distilled water (each three to four times the volume of the dialyzing solution) over a period of 72 hours. This solution of the dialyzed colloidal iron complex is quite stable.

*Preparation of Ammonium Aluminum-Sulfate Cochineal Solution.*—Powdered cochineal U. S. P., 30 gm.; ammonium aluminum sulfate, 26 gm.; distilled water, 450 cc.; ammonia 28%, 5 cc. (sufficient to give the solution the deepest purple-violet color).

Dissolve the ammonium aluminum sulfate in water, using heat if necessary, heat (or cool if heated) to 85 to 90 C. and add the powdered cochineal and stir thoroughly. Heat over a low flame, keeping the temperature of the solution within the range of 85 to 90 C. Add the ammonia gradually, 1 cc. at a time, and stir the precipitate formed until it dissolves. Do not allow the solution to boil while adding

2. These may be obtained from Visking Corporation, 6733 W. 65th St., Chicago.

ammonia. After the addition of ammonia the solution is boiled vigorously for 35 minutes. Add 100 to 150 cc. of distilled water, cool, filter and add a few crystals of phenol to prevent mold growth. This solution keeps well.

*Fixation.*—Four per cent neutral formaldehyde solution in water and 4% neutral formaldehyde solution in 90% alcohol are satisfactory fixatives for tissue sections which are to be stained by this method. Bichromate fixatives are not suitable because the bichromate oxidizes the polysaccharides and inhibits their combining with iron. The fixed tissue is dehydrated, cleared, and embedded in paraffin in the usual manner.

*Sectioning and Staining.*—1. Sections are cut at 5 to 7 $\mu$ and floated on a water bath adjusted to a temperature of not more than 38 C. Hotter water should be avoided; it melts the paraffin, and the polysaccharide may diffuse or dissolve. Affix sections to slides with egg-albumin and drain the water from the slide thoroughly. It is advisable to let slides stand for 10 minutes or longer before subjecting them to heat. Wet slides should not be heated, as this also may cause solution and diffusion of the polysaccharide.

2. Deparaffinize and hydrate the sections in the usual manner, passing slides through xylol, absolute, 95% and 80% alcohol into a 3% aqueous solution of acetic acid.

3. Stain for 10 minutes in an acid-colloidal complex iron solution made up as follows: stock dialyzed colloidal complex of ammonium ferric glycerate, 4 parts; concentrated glacial acetic acid, 1 part. (This solution is stable for one or two days.)

4. Wash slides thoroughly in distilled water five to six times until sections are colorless.

5. Immerse slides for 10 minutes in a solution of the following composition: 2% potassium ferrocyanide, 1 part; 1% hydrochloric acid, 2 parts. (Make up separate stock solutions of 2% potassium ferrocyanide and 1% hydrochloric acid and mix before using.)

6. Rinse slides twice in distilled water.

7. Stain in the ammonium aluminum-sulfate cochineal solution for 15 to 20 minutes.

8. Rinse in tap or distilled water to remove excess stain.

9. Stain in a picrofuchsin solution for 6 to 7 minutes. This is made up of 95 cc. saturated aqueous solution of picric acid (trinitrophenol) and 6 cc. of 1% acid fuchsin.

10. Without washing, pass quickly to 95% alcohol, followed by two changes of absolute alcohol, clear in toluol (or xylol) and mount in "permount"[3] or an oxidized solution of balsam in turpentine.

Nuclear chromatin stains a delicate brownish gray, collagen red and mucopolysaccharides bright blue. Mucoproteins, fibrin, and glycoproteins are stained buff or orange-colored; the basement membrane of the glomerulus stains in this manner. The cytoplasm of the cells usually stains greenish yellow to orange.

3. "Permount" is made by Fisher Scientific Company, Pittsburgh.

We have found that with certain modifications the acid mucopolysaccharide stain can be combined with the periodic acid, Schiff reagent technique devised by McManus [4] and Lillie.[5] The modified method follows:

After step 5 as set forth above, the slides are rinsed in distilled water followed by 70% alcohol and placed in 0.5 periodic acid in 70% alcohol for eight minutes. Following this the slides are rinsed in 70% alcohol and transferred to an alcoholic reducing rinse for 20 seconds. (Prepare this as follows: absolute alcohol, 60 cc.; potassium iodide, 2 gm.; sodium thiosulfate, 2 gm.; distilled water, 40 cc., and normal hydrochloric acid, 2 cc.) Slides are then rinsed in 70% alcohol and transferred to an alcoholic Schiff's reagent. (Prepare this as follows: 60% alcohol, 200 cc.; basic fuchsin, 1 gm.; sodium bisulfite, 3 gm., and normal hydrochloric acid, 20 cc. Dissolve the basic fuchsin in the 60% alcohol, filter and add sodium bisulfite and hydrochloric acid. Keep in dark for a few days before use.) From the Schiff's reagent transfer to an alcoholic sulfurous acid rinse—two changes of four minutes each. (The sulfurous acid rinse is made up as follows: 10% sodium metabisulfite—$NA_2S_2O_5$—6 cc.; normal hydrochloric acid, 5 cc., and 70% alcohol, 100 cc.) The slides are then stained for two minutes in Harris' hematoxylin diluted with an equal part of absolute alcohol. Wash in tap water for a few minutes and dehydrate in 95%

4. McManus, J. F. A.: The Periodic Acid Routine Applied to the Kidney, Am. J. Path. **24**:643, 1948.

5. Lillie, R. D.: Histopathologic Technic, The Blakiston Company, Philadelphia and Toronto, 1948, pp. 188-189.

EXPLANATION OF FIGURE

*A*, human skin showing the blue-staining acid mucopolysaccharides in the subepithelial reticular tissue, about the skin appendages and between collagen bundles; ×65.

*B*, sex skin of the rhesus monkey showing excessive accumulation of acid mucopolysaccharide in the corium; × 80.

*C*, segment of the sex skin of the rhesus monkey into which hyaluronidase has been injected in vivo. At the site of injection the skin pits readily on pressure. The mucopolysaccharide has largely disappeared; × 80.

*D*, essentially normal glomerulus. Note the orange-yellow coloration of the basement membrane and the blue-staining material on the surface; × 200.

*E*, glomerulus from a case of diabetes showing "glomerulosclerosis." Note the prominent thickening of the basement membrane. The surface epithelial cells show blue cytoplasm which surfaces the outer side of the basement membrane; × 200.

*F*, segment of the renal medulla. The blue-staining acid mucopolysaccharide occupies the interstices between the tubules. The relatively small amount of visible collagen stains red; × 130.

*G*, normal aorta from a rhesus monkey. Note the metachromatic staining of the intima and of the mucoid material immediately surrounding the elastic tissue fibers in the media. Toluidine blue; × 160.

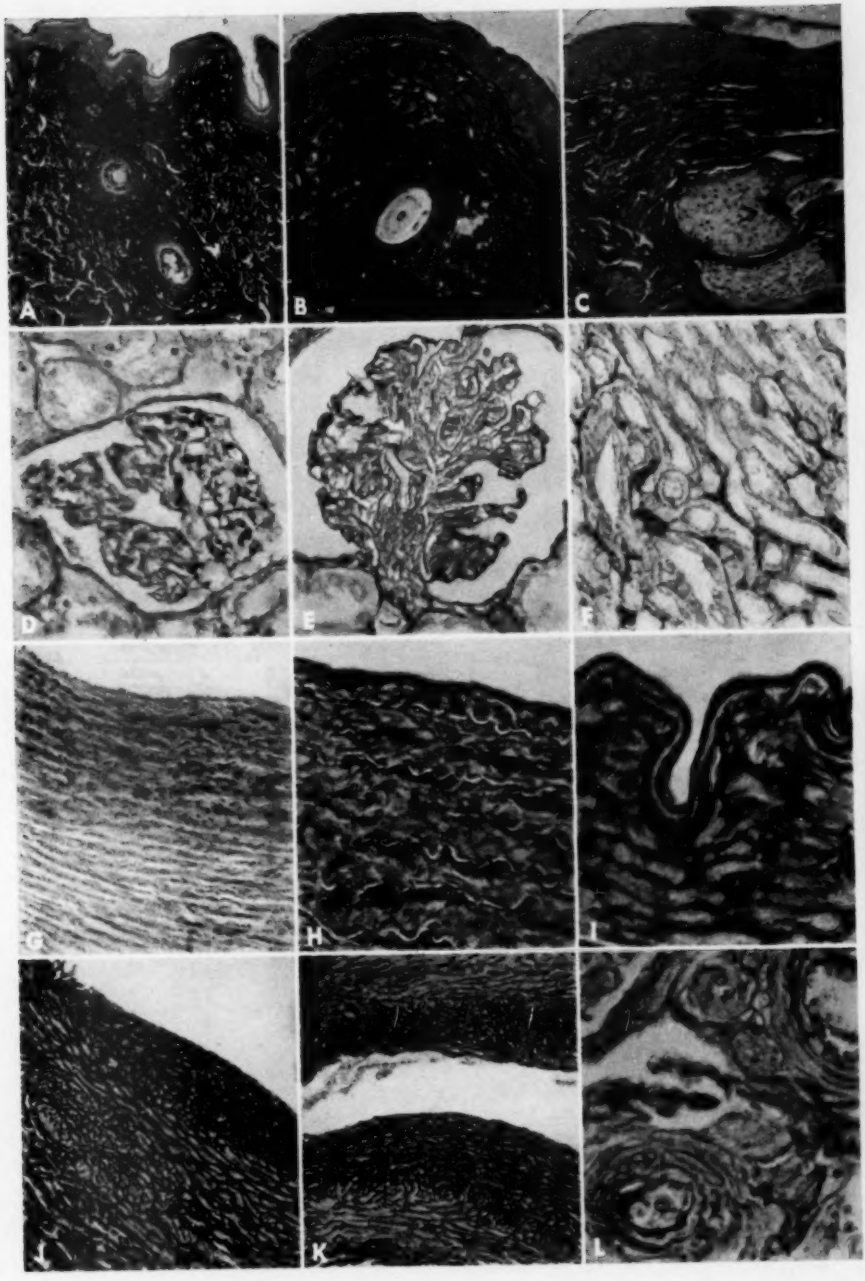
*H*, normal aorta from a rhesus monkey stained by the colloidal iron technique. The blue-staining acid mucopolysaccharide is distributed similarly to the metachromatic material; × 300.

*I*, branch of a human renal artery. The blue mucopolysaccharide will be noted in the intima and between muscle cells in the media; ×330.

*J*, sclerotic iliac artery in a pyridoxine-deficient monkey. A thick plaque of acid mucopolysaccharide has accumulated in the intima, and there is some excess of this substance between muscle fibers in the media. The collagen of the adventitia stains red; ×65.

*K*, human coronary artery with relatively early coronary sclerosis. The lesion is essentially similar to that shown in *J*; × 65.

*L*, sclerotic vessels in the kidney from a case of essential hypertension. Excess mucopolysaccharide is present in all layers of the artery showing hyperplastic sclerosis. The arteriole above contains a small amount of such material; × 260.


A
B
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and absolute alcohol. When this technique is utilized with alcoholic solutions, most of the acid mucopolysaccharide retains the blue color. The glomerular basement membrane is sharply delineated, staining reddish violet.

## APPLICATION OF THE METHOD

The general distribution of acid mucopolysaccharides in mammalian tissues has been recently described by Bunting,[6] who utilizes the metachromatic reaction given by toluidine blue. The method described here is believed to demonstrate more readily the finely distributed acid mucopolysaccharide and, in addition, possesses the advantage of clear counterstaining of collagen and differentiation of basement membranes.

The subepidermal connective tissue is relatively rich in acid mucopolysaccharide. In the guinea pig such material is present chiefly about the hair follicles, appearing as a delicate blue fibrillar mesh. We have found that this material is removed at sites where hyaluronidase is injected. While the acid mucopolysaccharide is probably not fibrillar itself, it may appear so because it is intimately related to finely dispersed collagen or reticulum fibers. Human skin shows considerable acid mucopolysaccharide. It is present in the subepidermal reticulum layer, about skin appendages and between collagen bundles (*A* in figure). As noted by Duran-Reynals and associates,[7] the sex skin of the rhesus monkey shows remarkable accumulations of an acid mucopolysaccharide, which is probably hyaluronic acid (figure, *B*). This substance is readily removed by in vivo or in vitro treatment with hyaluronidase (figure, *C*). It should be pointed out that the epithelial mucins also stain blue with the method described.

The renal glomerulus has long presented a difficult problem of histologic analysis. The staining method described appears to clarify the problem of the finer structure of glomerulus and offers considerable promise in the study of pathologic processes involving this structure. The wall of the glomerular loop is composed of a hyaline basement membrane which appears to represent a cytoplasmic extension of the endothelial cell. In well fixed tissue this substance stains an orange-yellow color contrasting sharply with the red-staining property of collagen. The epithelial cells which lie on the surface of the loop show bluish green cytoplasmic staining, and a delicate layer of similar staining material covers the surface of the glomerular basement membrane. Judged by the staining property and distribution, this substance would appear to be an acid mucopolysaccharide which is a product of the glomerular epithelium. It is not, however, removed by treatment with hyaluronidase. A normal glomerulus is shown in *D* in the figure, and *E* shows a glomerulus from a case of diabetic glomerulosclerosis. It appears that the major change in the diabetic glomerular loop is a thickening of the basement membrane and not a collagenous sclerosis—collagen staining bright red by the technique described. There is a considerable amount of acid mucopolysaccharide in the stroma of the renal pyramids which is well demonstrated by the technique presented. Here the material presents as a deep blue matrix, and it is sharply differentiated from the red-staining collagen

6. Bunting, H.: The Distribution of Acid Mucopolysaccarides in Mammalian Tissues as Revealed by Histochemical Methods, Ann. New York Acad. Sc. **52**:977, 1950.

7. Duran-Reynals, F.; Bunting, H., and van Wagenen, G.: Studies on the Sex Skin of Macaca Mulatta, Ann. New York Acad. Sc. **52**:1006, 1950.

(figure *F*). This mucopolysaccharide is readily removed by treatment of the section with hyaluronidase, leaving a very delicate fibrillar matrix of collagen in the background.

While a detailed analysis of the structure of blood vessels will be the subject of a subsequent report, the mucopolysaccharides constitute such an important element of the arterial structure and appear to be so intimately concerned in the pathogenesis of arteriosclerosis that the general relationships will be briefly reviewed. In the normal aorta a small amount of acid mucopolysaccharide is present in the intima and bathes the elastic tissue fibers of the media. In favorable material the acid mucopolysaccharide is readily demonstrated by the metachromatic staining reaction with toluidine blue (figure, *G*). A similar segment of tissue stained by the method detailed here shows a similar distribution of the mucopolysaccharide (figure, *H*). It would appear that the elastic tissue fibers can glide in this mucinous matrix and that it acts as an effective cement. It also seems likely that the mucopolysaccharide is a mother substance from which collagenous and elastic tissue fibers are formed. In the muscular arteries a small amount of the acid mucopolysaccharide is present in the intima and frequently is demonstrable in the media as shown in *I* in the figure. Some of this material is removed in sections pretreated with hyaluronidase. We have recently described the excessive accumulation of mucopolysaccharide encountered in human arteriosclerosis and in experimental arteriosclerosis which develops in the rhesus monkey subject to protracted pyridoxine deficiency.[8] *J* in the figure shows such a lesion in the iliac artery of a deficient animal. *K* shows an essentially similar lesion in a human coronary artery. Excess mucoid material in the wall of a small sclerotic artery and arteriole of the kidney from a case of essential hypertension is shown in *L*.

SUMMARY

A simple method of staining the mucoid ground substance and other elements of connective tissues is described and illustrated. The method appears to offer promise in the elucidation of the normal structure and relationships of the elements of connective tissue and in the analysis of alterations encountered in disease, particularly in arteriosclerosis and in renal and rheumatic diseases.

8. Rinehart, J. F., and Greenberg, L. D.: Pathogenesis of Experimental Arteriosclerosis in Pyridoxine Deficiency, with Notes on Similarities to Human Arteriosclerosis, Arch. Path. **51**: 12 (Jan.) 1951.

# A SIMPLE DISPOSABLE APPARATUS FOR DEHYDRATION OF FROZEN TISSUES

ROBERT B. JENNINGS, M.D.
CHICAGO

The advantages of the Altmann-Gersh [1] method of fixing tissue for histochemical study are well known. Through use of this method, tissue sections can be obtained which have not been exposed to the effects of solvents or chemical fixation. Inorganic salts, proteins, enzymes, and other substances all remain in the tissue in approximately their natural relationships and concentration.

This technique of drying tissue while it is frozen is in use in this laboratory in a study of the cytological localization of certain radioactive elements of long half life. A simple, inexpensive apparatus, which could, if necessary, be disposed of, was needed for dehydration of small pieces of radioactive tissue. The following design fulfills these qualifications and has proved to be eminently satisfactory.

## MATERIALS

The apparatus is constructed of pyrex® glass, and the connections are made through 15-mm. glass and 1 1/16-inch rubber vacuum tubing. It consists of two parts: a dehydration unit, and a unit for maintaining and measuring the vacuum.

The dehydrating unit (figure) is a 2-l. pyrex® Florence flask insulated with fiberglas® (*A*) and containing a centrally placed, horizontal vacuum tube, 30 mm. in diameter (*B*). This tube is surrounded by a mixture of dry ice and ethyl oxalate as described by Mendelow and Hamilton.[2] As dry ice-ethyl oxalate mixtures freeze at —40 C., the temperature of the tissue during the dehydration is easily maintained at levels below the minimum level of —30 C. recommended by Simpson.[3] For dehydrations at lower temperatures,[4] dry ice-organic solvent mixtures such as acetone-dry ice may be used.

Admission to the vacuum tube is through a 34 by 45-mm. tapered glass joint (*C*) which has a 3-mm. air-inlet stopcock (*D*) on its 9-cm. arm. If anhydrous $P_2O_5$ is used as a dehydrating agent, it may be placed in a combustion boat beneath the tissue or in the arm of the 34 by 45-mm. joint (*E*). The vacuum tube is connected through glass and rubber tubing to a trap (*F*).

The vacuum unit consists of a Welch® Duo-Seal mechanical pump, Model 1400 B (I), a pyrex® glass trap (*F*), and a Pirani Gage (*H*) with a range of 0.5 to 500 μ. When the Dewar flask (*G*) which surrounds the trap is filled with liquid nitrogen, water vapor passing from the specimen to the pump is frozen on the trap wall. Rapid dehydration can be assured through this use of liquid nitrogen.

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From the Department of Pathology, Northwestern University Medical School.

1. Gersh, I.: The Altmann Technique for Fixation by Drying While Freezing, Anat. Rec. **53**:309, 1932. Gersh, I.: Application in Pathology of the Method of Fixation by Freezing and Drying Tissues, Bull. Internat. A. M. Mus. **28**:179, 1948. Stowell, R. E.: A Modified Freezing-Drying Apparatus, J. Nat. Cancer Inst. **10**:1361, 1950.

2. Mendelow, H., and Hamilton, J. B.: A New Technique for Rapid Freezing and Dehydration of Tissues for Histology and Histochemistry, Anat. Rec. **107**:443, 1950.

3. Simpson, W. L.: An Experimental Analysis of the Altmann Technique of Freezing Drying, Anat. Rec. **80**:173, 1941.

4. Packer, D. M., and Scott, G. W.: A Cryostat of New Design for Low Temperature Tissue Dehydration, J. Tech. Methods, and Bull. Internat. A. M. Mus. **22**:85, 1942. Wang, K. J., and Grossman, M. I.: A Simplified Vacuum Dehydration Technique for the Preparation of Sections by Freezing-Drying, J. Lab. & Clin. Med. **34**:292, 1949.

PROCEDURE

Tissue is removed as rapidly as possible from the experimental animal, frozen with agitation in isopentane at liquid-nitrogen temperatures (—195 C.)[5] and placed in the dehydration unit. The vacuum tube and tissue are maintained at a temperature of —40 to —44 C. through the use of ethyl oxalate and dry ice. Although not essential, it has been found convenient to place the dehydration unit inside a deep-freeze refrigerator at a temperature of —20 to —26 C. in order to reduce sublimation of the dry ice. The vacuum tubing is connected to the liquid-nitrogen trap through a hole in the side of the refrigerator.

The vacuum is maintained at levels of 6 to 10 μ. This level has been found to be satisfactory and can be easily attained with a mechanical pump. Higher levels of vacuum can be obtained in this type of system only by using diffusion pumps in addition to the mechanical pump. Therefore, a diffusion pump is necessary if the dehydration is to be carried out in the lowest temperature range that is practical for this technique which is around —66 C.[6]

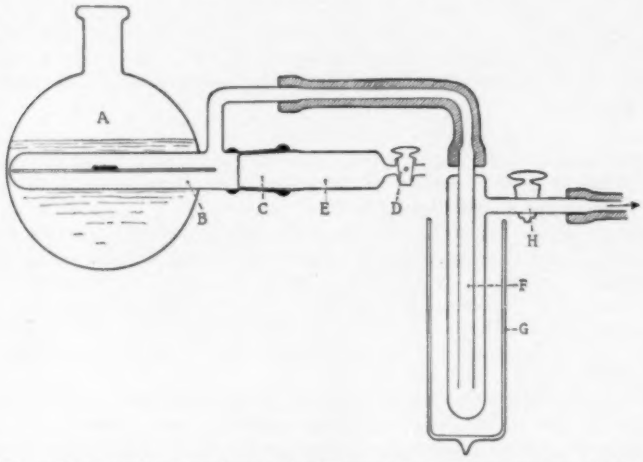


Diagram of freeze-drying apparatus: *A*, 2-l., Florence flask. *B*, vacuum tube. *C*, 34 by 45-mm. tapered glass joint. *D*, 3-mm. air-inlet stopcock. *E*, arm of tapered glass joint. *F*, line trap. *G*, Dewar flask. *H*, stopcock to Pirani gage.

The time required for complete dehydration of the tissue can be determined only empirically. In general, it is advisable to use small pieces of tissue. A section of liver or kidney 3 by 1 by 1 mm. can be dried easily in eight hours, whereas a section 5 by 3 by 10 mm. may require 48 to 72 hr.

When the tissue has been completely dehydrated, the vacuum is broken and the tissue brought to room temperature in a desiccator. Depending on its ultimate use, it can be vacuum-embedded in paraffin, denatured in absolute alcohol, or cut freehand.

Tissue containing radioactive inorganic elements, such as P32 and I131, is vacuum-embedded in paraffin. Thin, flat sections for radioautographs are obtained through the use of the paraffin-

5. Hoerr, N. L.: Cytological Studies by the Altmann-Gersh Freezing Drying Method: 1. Recent Advances in Technique, Anat. Rec. **65**:293, 1936.

6. The vapor pressure of water ice at —40 C. is $9.66 \times 10^{-2}$ mm. Hg. The pressure maintained routinely in this system is $9 \times 10^{-3}$ mm. Hg, which is well below the limiting pressure of $9.66 \times 10^{-2}$ mm. Hg. At —62.2 C. the vapor pressure of water ice is $6.5 \times 10^{-3}$ mm. Hg. Higher vacuums and increased pumping capacity are necessary to carry out dehydrations at this temperature.

backing technique,[7] in which the block is coated with a thin layer of paraffin, the section cut two to four seconds later, and the backed section applied to an albuminized slide. The disintegration of the dehydrated sections in water, plus the water solubility of many of the elements being studied, prevents the use of the ribbon flotation technique. Photographic film is placed in apposition with the tissue and radioautographs are taken after the excess paraffin has been melted off in a paraffin oven. After exposure, the tissue is denatured, stained, and correlated with the developed photographic plate.

SUMMARY

A description of an inexpensive apparatus for dehydration of frozen tissue is given. Included is a brief outline of the technique used in this laboratory in preparing tissue for radioautography.

7. Holt, M. W.; Cowing, R. F., and Warren, S.: Preparation of Radioautographs of Tissues Without Loss of Water Soluble P32, Science **110**:328, 1950. Mendelow and Hamilton.[2]

# Books Received

CYTOLOGY OF THE HUMAN VAGINA. By Ines L. C. de Allende, M.D., chief of the division of endocrinology, Mercedes and Martin Ferreya Institute of Medical Investigation, and Oscar Orias, M.D., director of the Mercedes and Martin Ferreyra Institute of Medical Investigation, Cordoba, Argentina. With a foreword by Bernardo A. Houssay, M.D. Translated from the Spanish by George W. Corner, M.D. Pp. 286, with 75 illustrations and 23 tables. Price $7.50. Paul B. Hoeber, Inc. (Medical Book Department of Harper & Brothers), 49 E. 33d St., New York 16; 90 Great Russell St., London, W.C.1, 1950.

The cytologic method of Papanicolaou and Shorr for the study of the sex cycle of the mammalian female is here applied to the human in the first major endeavor of its kind. Previous reports by other authors have emphasized the inconvenient variability of the vaginal smear in the human cycle as compared with that of the lower mammals and have attempted to find some standard means of correlating the characteristics of the vaginal smear with hormone activity. Several types and subtypes have been used by Papanicolaou and Traut in an attempt to classify smears, and additional subtypes have been proposed by Kernodle and Cuyler, so that a rather cumbersome series of possibilities and combinations of obscure significance has resulted.

Drs. de Allende and Orias propose an actual "differential count" of the cell types in vaginal smears and demonstrate that linear graphing of these counts with daily smears through one or two complete cycles can reveal the various degrees and forms of endocrine disturbance. They present data of this type for a large number of normals, thus establishing fairly well the range of normal variation in "cytological curves," and have investigated smaller but appreciable numbers of cases of amenorrhea, sterility, and hemorrhagic metropathy. Consideration has also been given to the effect of hormone therapy on the menopause and other conditions and on the anovulatory cycle. The contrasting cell type curves presented in these studies are impressive evidence that this method may offer a far more concise means of diagnosis and guidance in endocrine problems than has heretofore been available.

The Shorr stain has been chosen by these authors for its simplicity and vivid differentiation of squamous epithelial types, and some minor modifications in the technique of preparing the smears have been suggested. The book is prolifically illustrated with excellent colored photomicrographs and with graphs and tables which, while lucid, are rather inconveniently printed in the long axis of the page making it necessary to turn the book to read them. The graphs particularly are not sufficiently complicated to require this much space.

ATLAS OF HISTOLOGIC DIAGNOSIS IN SURGICAL PATHOLOGY. By Karl T. Neubuerger, M.D., professor of pathology, University of Colorado School of Medicine. With a Section on Exfoliative Cytology by Walter T. Wikle, M.S., M.D., assistant professor of pathology, University of Colorado School of Medicine. Pp. 460, with 880 illustrations. Price $11. Williams & Wilkins Company, Mount Royal and Guilford Aves., Baltimore 2, 1951.

There is nothing that can serve as a substitute for a good collection of slides, a worthy microscope, and an enthusiastic instructor in the teaching of the histologic aspects of surgical pathology. That being the ideal, atlases of surgical pathology do have their place. To achieve their objective, however, the illustrations should be as nearly perfect as possible and, were it not that price is a consideration, preferably in color. The present atlas falls somewhat short in that regard. Many of the photomicrographs are so dark and at such low magnification that it is hard to see how they can be instructive in any way. Furthermore, they bear with them the marks of distortion and artefacts not uncommon in surgical biopsies. In the aggregate, however, the student will find in the illustrations of this atlas, the main features of any specified lesion. The subject matter is well covered, and the brief descriptions accompanying the photographs are highly informative.

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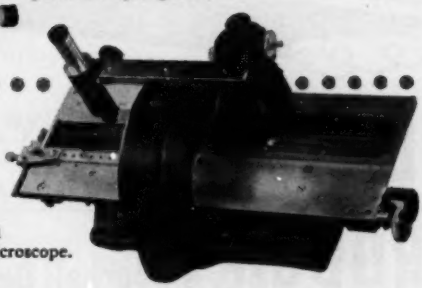

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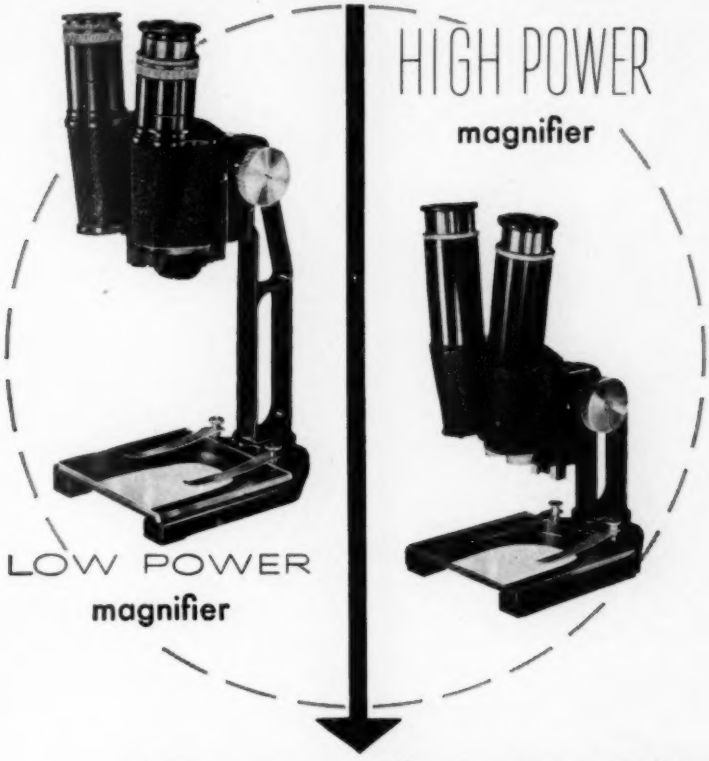


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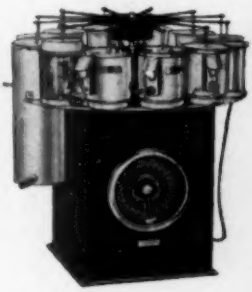

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